Characterisation of Chemical Components for Identifying Historical Chinese Textile Dyes by Ultra High Performance Liquid Chromatography - Photodiode Array - Electrospray Ionisation Mass Spectrometer

Jing Han[a,*] , Jantien Wanrooij[b], Maarten van Bommel[b,c], Anita Quye[a]

[a] Centre for Textile Conservation and Technical Art History, School of Cultural and Creative Arts, University of Glasgow, Glasgow G12 8QH, United Kingdom

[b] Cultural Heritage Agency of the Netherlands, Hobbemastraat 22, 1071 ZC Amsterdam, The Netherlands

[c] University of Amsterdam, Conservation and Restoration of Cultural Heritage, Johannes Vermeerplein 1, 1071 DV Amsterdam, The Netherlands

Highlights

- Novel application of UHPLC-PDA-MS to the chemical characterisation of Chinese dyes.
- Improved combination of three extraction methods for sample preparation.
- First LC-PDA-MS reference database of common historical Chinese dyes.
- Better understanding of gallotannins in gallnut dye, and crocins in gardenia and saffron dyes.
- First report of 6-hydroxyrubiadin in a Chinese *Rubia cordifolia* dyed sample.

ABSTRACT

This research makes the first attempt to apply Ultra High Performance Liquid Chromatography (UHPLC) coupled to both Photodiode Array detection (PDA) and Electrospray Ionisation Mass Spectrometer (ESI-MS) to the chemical characterisation of common textile dyes in ancient China. Three different extraction methods, respectively involving dimethyl sulfoxide (DMSO)-oxalic acid, DMSO and

* Corresponding author. Tel.: +44 (0) 141 330 5467.
E-mail address: jinghan8706@hotmail.com (J. Han); Jantien.wanrooij@gmail.com (J. Wanrooij); M.R.vanbommel@uva.nl (M. van Bommel); anita.quye@glasgow.ac.uk (A. Quye);

hydrochloric acid, are unprecedentedly applied together to achieve an in-depth understanding of the chemical composition of these dyes. The first LC-PDA-MS database of the chemical composition of common dyes in ancient China has been established. The phenomena of esterification and isomerisation of the dye constituents of gallnut, gardenia and saffron, and the dye composition of acorn cup dyed silk are clarified for the first time. 6-Hydroxyrubiadin and its glycosides are first reported on a dyed sample with *Rubia cordifolia* from China. UHPLC-PDA-ESI-MS with a C18 BEH shield column shows significant advantages in the separation and identification of similar dye constituents, particularly in the cases of analysing pagoda bud and turmeric dyed sample extracts.

Keywords: Historical Chinese dyestuffs, dye components, UHPLC-PDA-ESI-MS, extraction methods, textiles, cultural heritage

1 Introduction

In ancient China colour held an important role, both symbolising social ranking and conveying rich cultural meanings. The identification of dyes on historical Chinese costume and textiles not only reveals how the specific colours of importance were obtained but also assists in determining the provenance and guiding preservation efforts of these costume and textiles. There has been extensive chemical research on historical dyes over the past several decades [1]. In the aspect of Chinese and Asian dyes, research has been carried out to identify historical and archaeological dyes [2-7], and to characterise in detail the chemical composition of dyes of specific groups such as flavonoids, protoberberines and *Rubia* species [8-11]. However, fundamental research on the detailed chemical characterisation of dyes in ancient China is still very limited. Faced with increasing demands for the robust identification of historical and archaeological dyes, this research investigates the chemical composition of twelve common dyes in ancient China and establishes the first database of their chemical profiles.

Liquid chromatography combined to UV-vis spectrometry and mass spectrometry has increased the capacity to identify chemical substances in different fields [12-14]. The use of UV-Vis spectra to identify chemical components has some uncertainties, e.g. UV-vis absorption of chemical components is influenced by the mobile phase, and it is difficult to differentiate components with similar UV-vis absorption due to the lack of fine spectral details. Mass spectrometry analysis provides more detailed molecular structural information, i.e. the mass-to-charge ratio of the fragmental ions of the components, and thus ensures more reliable identification. This research makes the first attempt to apply Ultra High Performance Liquid Chromatography (UHPLC) coupled to both Photodiode Array detection (PDA) and Electrospray Ionisation Mass Spectrometer (ESI-MS) to the chemical characterisation of dyes commonly used in ancient China. This methodology utilises the high resolution of UHPLC. Improved separation power is achieved using columns packed with very small particles (1.7 μm diameter) as the stationary phase, allowing the separation of structurally similar components. Moreover, UHPLC-PDA enables a lower limit of detection (LOD), i.e. the LOD of selected common colourants in historical dyestuffs can be as low as 0.02 μg/mL [15]. This is of great importance for the chemical analysis of cultural heritage objects as the samples are very precious and the amount of sample available for destructive analysis is usually very limited. Combined, the benefits of improved sensitivity and resolution of UHPLC coupled with appropriate columns and detectors prove to be key in helping to distinguish and identify trace components. The result is more comprehensive knowledge of dye compositions, which provides a solid foundation for further investigations into dye sources and dyeing procedures, and the preservation of the dyed textiles.

An improved combination of three extraction methods was used during the sample preparation stage, which involved application of dimethyl sulfoxide (DMSO)-oxalic acid (OA), DMSO and hydrochloric acid (HCl) respectively to dyed silk and dyes, allowing in-depth characterisation of their chemical compositions . DMSO is especially suitable for dyes which bind to the textile fibre via hydrophobic

interactions, i.e. vat dyes and direct dyes, while OA and HCl are used to break the metal-dye bond of mordant dyes. DMSO-OA and DMSO are considered mild extraction methods, preserving the sugar bonds of dye components. HCl is considered a harsh method, breaking down the fabric-dye and metal-dye bonds very effectively, but this method also breaks down the sugar bonds and causes possible changes to some components through hydrolysis, decarboxylation and esterification [16].

The chemical characterisation of common historical Chinese dyes greatly enhances the analytical methodologies of identifying dyes on historical and archaeological textiles, and significantly contributes to the better interpretation and preservation of the dyed textiles, including textiles not only from China but also from other geographical areas where similar dyes were used.

2 Materials and Methods

2.1 Materials

This research studied twelve common dyes used in ancient China [7]. Most dyes collected to prepare the reference dyed samples are of reliable botanical provenance [17]. Safflower (*Carthamus tinctorius* L., root), sappanwood (*Caesalpinia sp.*, most likely *Caesalpinia sappan* L., heartwood and larger branches), gromwell (most likely *Lithospermum sp*, root), Chinese cork tree (*Phellodendron chinense* Schneid., bark), turmeric (*Curcuma longa* L., rhizome), pagoda bud (*Styphnolobium japonicum* (L.) Schott, bud), gardenia (*Gardenia jasminoindes f. longicarpa* Z. W. Xie & M. Okada, fruit), indigo (*Strobilanthes cusia* (Nees) Kuntze*, Persicaria tinctoria* (Aiton) H.Gross*, Indigofera tinctoria* L. or *Isatis tinctoria* L., leaf) and gallnut (produced by the insect *Melaphis chinensis* Bell or *M. paitan* Tsai et Tang) were purchased in Chinese medicine shops in Anguo, Beijing and Shanghai. Acorn cup (*Quercus acutissima* Carr. or *Quercus wutaishanica* Mayr.) was collected at Peking University. As there are several similar species to munjeet (*Rubia cordifolia* L., root, also known as Indian madder) and smoketree (*Cotinus coggygria var. chinerea* Engl., wood) in China, plant samples of correct species were collected respectively from a hill in

Beijing and the botanical garden at the Institute of Medicinal Plant Development, Beijing, by botanists.

Pure chemicals were used as dyeing additives to identify the key components of reference dyes before analysing chemically complex historical dye samples. The chemicals used included aluminium potassium sulphate dodecahydrate and sodium carbonate from Sigma-Aldrich (Dorset, UK); iron (II) sulphate, potassium carbonate, sodium hydroxide and citric acid monohydrate from Acros organics (Geel, Belgium); acetic acid and ethanol from Fisher Scientific (Loughborough, UK); and thiourea dioxide from Fibrecrafts (Guildford, UK).

For sample preparation, DMSO, oxalic acid dihydrate and 37% HCl from Merck-Schuchardt (Hohenbrunn, Germany), and methanol from Sigma-Aldrich (Munich, Germany) were used. For the preparation of eluents for UHPLC analysis, methanol and formic acid from Sigma-Aldrich (Munich, Germany) and deionised water (Millipore Simplicity TM Simpak® 2, R = 18.2 MΩ·cm, Ettenleur, The Netherlands) were used.

2.2 Dyeing

Historical Chinese dye recipes, especially those using a single dye, were replicated to dye reference silk samples [7]. Detailed recipes and procedures are presented in Table A.1.

2.3 Analytical Methods

Extraction. Three different extraction methods (DMSO-OA, DMSO and HCl) were applied to dyed silk and dyes. . Since reference materials were used, the sample size was increased to 1 mg so that as many major and minor components as possible could be detected and characterised. Samples from cultural heritage objects can be as light as 50 μg.

Extraction method 1. The two-step extraction method using DMSO and oxalic acid was applied to samples from all the dyed silk fabrics and an undyed silk fabric.

Approximately 1 mg of dyed silk was weighed with a microbalance and transferred into a 1 ml flat-bottom glass vial. 100 µl of DMSO was added with a micropipette and then the vial was heated at 80 °C for 10 minutes in a water bath. Next, the DMSO extract was transferred by a micropipette with a disposable tip into a 300 µl vial insert and this extract retained. An aliquot of 100 µl of oxalic acid solution (0.5 M oxalic acid / acetone / water / methanol, 1:30:40:30 (v/v/v/v)) was added to the fibre sample remaining in the vial. The sample was heated for a further 15 minutes at 80 °C in the water bath and then the extract was evaporated to dryness using a gentle nitrogen flow. This dried extract was reconstituted using the first DMSO fraction, thereby combining the extracts from the two steps.

Extraction method 2: munjeet. The munjeet dyed silk was extracted with 100 µl methanolic hydrochloric acid solution (6 N HCl / water / methanol, 1:1:2 (v/v/v)) at 100 °C for 10 minutes. The sample was then evaporated to dryness and reconstituted with 100 µl DMSO.

Extraction method 3: gromwell. The dye components of gromwell were extracted directly from the dye using 100 µl DMSO, heated at 80 °C for 10 minutes.

The following step of the three extraction methods was to centrifuge the (combined) extracts for 10 minutes at 2000 rpm. The supernatant was transferred into a 250 µl micro-insert vial, with great care taken to avoid transferring any precipitates. These solutions were again centrifuged for 10 minutes at 2000 rpm to avoid the injection of remaining particles which could block the UHPLC column.

UHPLC analysis. A Waters ACQUITY UPLC H-Class system, composed of a quaternary solvent manager, a sample manager, a column manager and a PDA detector, all controlled by Empower Software, was used for the identification of dye components. A Waters C18 Ethylene Bridged Hybrid (BEH) shield column (150 × 2 mm I.D., particle size 1.7 µm) was installed for separation. A volume of 2 µl was injected for each analysis. A gradient elution programme published earlier involving water, methanol and formic acid and with the flow rate of 0.2 ml/min was used [15].

PDA detection. UHPLC-PDA analysis was applied to all the samples. UV-Vis data from 190 to 800 nm was collected with a resolution of 1.2 nm and the chromatogram was monitored at 254 nm. The characteristic components were identified by means of their UV-Vis spectra in combination with their retention time. A UHPLC-PDA software library at the Cultural Heritage Agency of the Netherlands was consulted, which contains more than 100 spectra of reference materials extracted and analysed under exactly the same conditions.

ESI-MS detection. The MS system used was a Micromass QTOF-2 system with an electrospray ionisation ion source inlet, a quadrupole and an orthogonal time-of-flight analyser, controlled by MassLynx NT software. ESI was chosen not only for the ease of coupling with LC, but also because this soft technique minimises fragmentation for targeted molecules and thus is suitable to detect the molecular weight of the analytes [18]. A split was used such that 20% of the effluent was transported to the MS detector while 80% of the effluent was guided through the PDA detector so that both data were obtained simultaneously. A negative ionisation mode was used. The collision energy was 8-10 eV for single MS mode. Source conditions included a capillary voltage of 3.0 kV, a cone voltage of 40 V, a source temperature of 80 °C and a desolvation gas temperature of 150 °C. The nitrogen gas flow rate was set at 120 L/h for cone gas, 90 L/h for nebulizer gas and 120 L/h for desolvation gas. The scan range for m/z was 0-800, but was adjusted to 0-1100 for the gallnut dyed sample. Published MS data of the dyes in the fields of cultural heritage, medicine, food chemistry and forestry science [4, 19-21] was consulted for data interpretation.

MS/MS. The collision energy was set first at 80 eV and increased to 160 eV when necessary.

3 Results and Discussion

Through UHPLC-PDA-MS analysis, some characteristic components not reported earlier on dyed textiles were clarified, especially for the dyes gallnut, acorn cup,

gardenia and munjeet. Components of similar chemical structures showed improved separation and were identified.

3.1 Gallnut

The UHPLC-PDA profile of the extract from the gallnut dyed sample (Fig. 1 and Fig. C.1) shows a series of constituents with similar UV-Vis absorption profiles and maximum absorption at around 219 and 277 nm. By consulting the in-house UHPLC-PDA library, gallic acid and ellagic acid were identified.

The MS analytical result (Fig. C.1) shows the m/z values of this series of components increase from 169 to 1243 Da, with intervals of 152 Da in between. Gallnut is mainly composed of gallotannins, i.e. polyphenol molecules formed by the esterification of a central β-D-glucose with surrounding gallic acid units (Fig. 2) [22]. By comparing with published data of the molecular weight of gallotannins from tannic acid and gallnut extracts [20, 23], the main components of this sample were respectively identified as gallic acid isomer, trigalloyl glucose, tetragalloyl glucose, pentagalloyl glucose, hexagalloyl glucose and heptagalloyl glucose, and a trace amount of dimer was detected (Table 1 and Fig. 2). Among a large amount of natural plants containing gallotannins, hexagalloyl glucose was only reported to be present in gallnut [24], and thus this component may be used as a marker component for gallnut.

The isomerisation of digalloyl glucose, trigalloyl glucose and tetragalloyl glucose was found. These isomers differ in the position where the galloyl groups are attached with the central β-D-glucose (Fig. 2). The broad peaks of hexagalloyl glucose and heptagalloyl glucose in the chromatogram probably indicate coelution of isomers [26]. No isomer of pentagalloyl glucose was found, probably because the attachment of one galloyl with each of the five hydroxyls of the central β-D-glucose forms the most stable structure. Some other ions were detected, including doubly charged ions of pentagalloyl glucose and heptagalloyl glucose, dehydrated galloyl glucose ions of

trigalloyl glucose and pentagalloyl glucose , decarboxylated gallic acid monomer ions , and anhydride ellagic acid ions .

The isomerisation and different degrees of esterification of gallotannins in dyed fabrics is clarified for the first time. The in-depth knowledge of gallotannins contained in gallnut dyed fabrics is a good starting point for the identification of dye sources for gallotannins in historical textiles, which is a major group of dye sources for dark shades.

3.2 Acorn cup

By comparing the UHPLC-PDA profile of the extract from the acorn cup dyed sample (Fig. C.2) with data in the UHPLC library, its two main constituents were identified as ellagic acid and its equivalent. The term 'equivalent' in this research refers to an unidentified component with an UV-Vis spectrum similar to that of an identified component but with different retention time. It is expected that the chemical structures of the two components are similar, with minor differences probably resulting from connected sugar moieties, esterification, polymerisation or isomerisation. In this case, the detected equivalent of ellagic acid is probably an ellagitannin, i.e. a pentagalloyl glucose with gallic acid units attached, and the gallic acid units connected with each other by oxidation (Fig. B.1) [22]. Different oxidation pathways of the gallic acid units, different binding positions of the gallic acid units with the central glucose, etc, result in isomers of ellagitannin [32]. Only one published report elucidated various ellagitannins in acorn cup, including isovalolaginic acid, vescalin, valolaginic acid, vescalagin, vescvalonininc acid, castalagin and castavaloninic acid [33]. Further investigations are needed to identify the ellagitannin eluting at 7.5 min. In addition, two gallotannins, namely pentagalloyl glucose and hexagalloyl glucose, were identified, as well as a gallotannin dimer which probably co-elutes with the component at 7.5 min, judging from the UV-Vis spectrum. This is the first time that the dye composition of acorn cup has been characterised, contributing to the

identification of dye sources containing ellagitannins, which is also a major group of dye sources for dark shades.

3.3 Gardenia

The UHPLC-PDA-ESI-MS profiles of the extract from the gardenia dyed sample (Fig. 3 and Fig. C.4) shows that all its major constituents have adjacent maximum absorption at around 426 and 464 nm, and molecular ions at m/z values of 327, 489, 651 and 813 Da were detected repetitively. By consulting the UHPLC library, crocetin was identified. Gardenia also contains a large number of crocins, which are the glycosyl esters of crocetin. By consulting published data of the m/z values, UV-Vis absorption profiles, eluting sequences and relative amounts of crocins in the commercial extract of *Gardenia jasminoides* Ellis fruits by HPLC-ESI-MS [21], the main components of the gardenia dyed sample extract were tentatively identified (Table 1 and Fig. 3). Molecular ions at m/z values of 327, 489, 651, 813 and 975 Da were respectively identified as crocetin, and crocins with 1, 2, 3 and 4 glucose units. The repetitive detection of these molecular ions results from the loss of glucoses from the crocin molecular ions, as well as the isomerisation of crocins, due to different distributions of glycosyls on the two ends of crocetin and *cis*-*trans* isomerisation (Fig. 2) [21].

Additionally, three chromatographic trends on the eluting sequence of various crocins were found. Firstly, for crocins of the same type of steric configuration (*cis* or *trans*), those with more glucosyls elute earlier, because the glucosyls improve the hydrophilicity of the molecules. Secondly, for crocins with the same number of glucosyls, *trans*-crocins elute earlier than *cis*-crocins. Thirdly, for crocins with the same number of glucosyls and of the same steric configuration (*cis* or *trans*), crocins with glucosyls equally distributed at the two ends elute earlier than crocins with unequally distributed glucosyls, e.g. *cis*-2gg-crocin elutes earlier than 2G-crocin. The latter two trends are probably because *trans*-crocins and crocins with equally

distributed glucosyls have less steric hindrance than their counterparts, and thus elute earlier. Differences in the UV-Vis absorption of *cis*- and *trans*- crocins found previously were also confirmed [21]: *cis*-crocins contain extra characteristic absorption at around 320-325 nm; the maximum absorption of *trans*-crocins is about 5-nm longer than that of corresponding *cis*-crocins.

Similar to gardenia, saffron (*Crocus sativus*), another important yellow dye in parts of Asia and Europe in ancient times, also contains abundant crocetin and crocins [1]. UHPLC-PDA analysis was undertaken on an extract from a silk sample directly dyed by saffron (saffron was purchased from a Chinese medicine shop in Beijing), and its main components were identified as *trans*-4-GG-crocin, *trans*-4-ng-crocin, *trans*-3-Gg-crocin and *cis*-4-GG-crocin (Fig. 3 and Fig. C.4).

The *cis-trans* isomerisation and esterification patterns of crocins in gardenia and saffron dyed silk extracts are clarified for the first time, significantly contributing to the differentiation of the two important dye sources in historical textiles.

3.4 Munjeet

By comparing the UHPLC-PDA profile of the DMSO-OA extract of munjeet (*Rubia cordifolia*) dyed silk (Fig. 4(a) and Fig. C.5) and data in the UHPLC library, lucidin-3-O-primeveroside, purpurin and alizarin were identified. Among the unidentified components, five components share similar UV-Vis maximum absorption, four of which are at 275 and 416 nm, and one at 278 and 429 nm (Fig. C.5), indicating these components have similar chemical structures, probably an aglycon and its glycosides. To confirm the presence of glycosides and identify these components, hydrochloric acid solvent was applied to sample preparation. Lucidin-3-O-primeveroside and five major components in the DMSO-OA extract disappear in the HCl extract (Fig. 4(b) and Fig C.5.), while the amount of a component eluting at 25.7 min in the HCl extract increases dramatically, indicating that this component may be an aglycone and those disappearing in the HCl extract may be its glycosides.

Analytical results by MS show that the m/z value of this component eluting at 25.7 min is 269 Da. Four anthraquinones with the molecular weight of 270 Da have been reported, respectively lucidin, 6-hydroxyrubiadin, anthragallol 3-methyl ether and 1,4-dihydroxy-2-hydroxymethyl-anthraquinone [11, 34]. By consulting chromatographic and spectral information provided by Mouri and Laursen, this component was identified as 6-hydroxyrubiadin (Fig. 5). This was further confirmed by the report of the presence of 6-hydroxyrubiadin and its sugars in *Rubia cordifolia* from China [35]. The analytical result of this component by MS/MS shows the presence of a fragment at m/z 239 Da. The loss of 39 Da is most likely due to the combined loss of one methoxyl group and one hydroxyl group.

Based on the identification of 6-hydroxyrubiadin and comparing the m/z values of the molecular ions, UV-Vis spectra and retention time (Fig C.5) with information provided by Mouri and Laursen and published data [24], the other main components were identified, including three 6-hydroxyrubiadin sugars and esters, an isomer, and rubiadin (Table 1). With the current gradient elution programme of water, methanol and formic acid, two main components of munjeet, namely munjistin and pseudopurpurin, both acids, are partly ionised, partly neutral, and thus these peaks do not resolve completely, resulting in broad peaks in the chromatogram.

The finding of 6-hydroxyrubiadin and its derivatives in *R. cordifolia* from China highlights a potential difference in chemical composition among *R. cordifolia* from various regions. *R. cordifolia* distributes over a large range of areas including Africa, tropical Asia, China, Japan and Australia [1]. Chemical characterisation of *R. cordifolia* from Bhutan, Tanakanao (uncertain in the original report) and Nepal did not show the presence of these components [11], rather, their presence was reported to be characteristic of *R. cordifolia*, *R. cordifolia* var. *pratensis* (now regarded as a synonom of *R. cordifolia*), *R. akane* and *R. oncotricha* [36-38]. The presence of 6-hydroxyrubiadin and its derivatives in *R. cordifolia* from China contributes to robust identification of *Rubia* species and their places of origin. Further work is needed to investigate the presence of 6-hydroxyrubiadin and its derivatives in *R.*

cordifolia, *R. akane* and other main *Rubia* species from different regions to ensure correct identification using this marker component.

3.5 Turmeric

For the turmeric and pagoda bud dyed silk, the improved methodology resulted in greater chromatographic resolution of their major components. The UHPLC-PDA result of the extract of the turmeric dyed silk shows the presence of three curcuminoids sharing similar UV-Vis absorption profiles with minor bathochromic shifts (differences within 10 nm in the maximum absorption wavelength) (Fig. C.6). The MS results show that the m/z value of the molecular ions are respectively 367, 337 and 307 Da, with a continual decrease of 30 Da, indicating the loss of a methoxy group. Therefore, the three main components of turmeric were identified as curcumin, desmethoxycurcumin and bisdesmethoxycurcumin (Table 1) . These three components usually co-elute in HPLC system because of their highly similar chemical structures [6]. The use of an UHPLC C18 BEH shield column enhances the separation, and thus leads to the identification, of these three components.

3.6 Pagoda bud

The chromatographic result of the extract from the silk sample dyed with pagoda bud shows six main components with maximum absorption at around 255 nm and 351-370 nm (Fig. C.7). By consulting the UHPLC library, rutin (also known as quercetin rutinoside), quercetin, isorhamnetin and kaempferol were identified. MS results show that the m/z values of the other two components are 623 and 593 Da, respectively 308 Da more than those of isorhamnetin and kaempferol, indicating that these two components are the rutinosides of the two aglycones (Table 1). The three rutinosides elute earlier because they are more hydrophilic. In addition, the UV-Vis absorption maxima of rutinosides shift to shorter wavelengths. In HPLC systems various rutinosides of pagoda bud usually co-elute, and kaempferol and isorhamnetin co-elute [6, 9]. This is the first successful separation of these dye components by LC method,

offering an analytical methodology for better knowledge of the dye components of pagoda bud in historical textiles.

3.7 Indigo

Although there are no new findings from the analytical results of the samples dyed by the other dyes in this study, for completeness of the database their data is presented. Isatin, indigotin and indirubin were identified in the extract from the indigo dyed sample (Fig. C.8). Characteristic constituents in different plant sources for indigo may be used as marker components to differentiate these plant sources [26], which would improve the understanding of indigo dyeing in ancient times. For example, 'pseudoindirubin' has been found in dyed samples and may lead to being a marker component for woad (*Isatis tinctoria* L.) [39], although additional research is required to confirm this hypothesis.

3.8 Chinese cork tree

The main component of the Chinese cork tree dyed sample was identified as berberine (Fig. C.9). Small amounts of equivalents of berberine are also present including magnoflorine, phellodedrine, palmatine and jatrorrhizine [24]. The characteristic alkaloid components and their relative amounts are distinguishing enough to differentiate dye sources of alkaloid in Asia [9].

3.9 Smoketree

The two main dye components of the smoketree dyed sample were identified as sulfuretin and fisetin (Fig. C.10). The extremely low peak area ratio of fisetin to sulfuretin (less than 0.1 in this research) in smoketree dyed samples may be characteristic to differentiate smoketree from young fustic (*Cotinus coggygria*), whose ratio is much higher (approximately 0.5-1.5) [28]. Smoketree also contains several additional compounds including sulfurein and disulfurein [28].

3.10 Sappanwood

The main dye component of sappanwood, brasilein, and its precursor, brasilin, were identified in the extract from the sappanwood dyed sample (Fig. C.11). Brasilein appears as a broad and tailing peak in the chromatogram. Some other brasilin derivatives and flavonoids identified in previous research may be present as well [1, 4]. Several colourless but characteristic components of sappanwood were found, namely Nowik Type A and Type C components, which are relatively lightfast and thus often used as marker components for sappanwood in historical textiles especially when its main dye components are degraded [40].

3.11 Safflower

Carthamin was identified in the extract from the safflower dyed sample (Fig. C.12). Four colourless components named as Ct1-Ct4 reported in previous research were also detected. Because of their stability to hydrolysis during extraction and their light fastness, these colourless components are used as markers for safflower in historical textiles especially when carthamin is degraded [30].

3.12 Gromwell

Shikonin and its equivalent were identified in the extract from the gromwell dyed sample (Fig. C.13). The main dye components in the roots of gromwell are S- and R-enantiomers (namely shikonin and alkannin) [41]. It is impossible to differentiate this pair of enantiomers by UHPLC-PDA-MS because they co-elute and they have no spectral differences [42]. Other analytical techniques like nuclear magnetic resonance have been applied to differentiate and identify various shikonin and alkannin [31, 41].

4 Conclusions

This research undertook novel application of UHPLC-PDA-ESI-MS and three different extraction methods to analyse the chemical composition of common dyes in ancient China. The characteristic components of these dyes were identified and an

UHPLC-PDA-MS database for historical Chinese dyes was established. The understanding of the chemical composition of these dyes was improved, including the phenomena of esterification and isomerisation of the dye constituents of gallnut, gardenia and saffron; and the dye composition of acorn cup. 6-Hydroxyrubiadin and its glycosides were first reported to be present in *Rubia cordifolia* dyed sample extracts. These research results form an important foundation for the identification and interpretation of dyes on historical and archaeological Chinese textiles and textiles from other geographical areas where similar dyes were used [7]. Further studies on changes in the composition of these dyes during dyeing and ageing processes will contribute to better identification of dyes. Investigations into the similarities and differences in chemical composition among dyes of the same species but from different regions and among dyes of similar species will enable better identification of provenance of the dye sources and dyed textiles.

The technique of UHPLC, used with a C18 BEH shield column and appropriate PDA and MS detectors, proved its advantage in enhancing the separation effect of similar components and in increasing detection limit, allowing successful identification of dye components, such as the main components of pagoda bud and turmeric, and the trace amounts of crocins present in the gardenia dyed sample extract. This shows the great potential of UHPLC for analysing cultural heritage objects. The combination of different extraction methods also greatly facilitated the identification of dye components.

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Appendices. Supplementary data

Supplementary materials related to this article including (A) dye recipes, (B) the chemical structure of the dye components, and (C) chromatograms, UV spectra and MS spectra that are not included in the main text.

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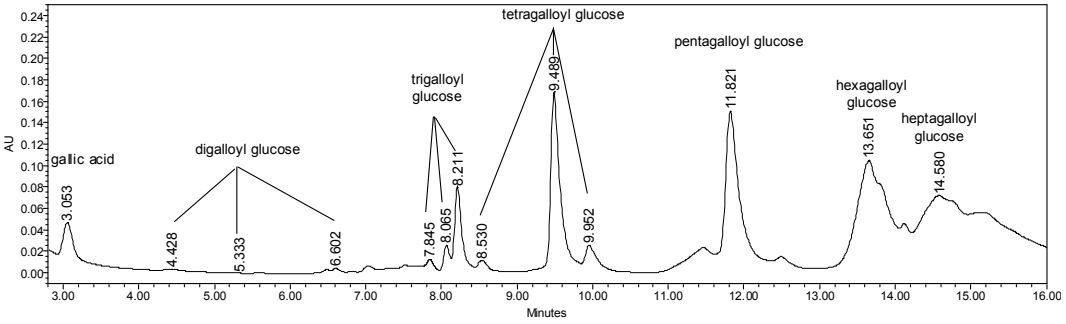
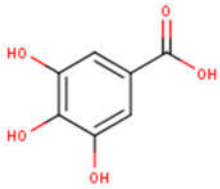
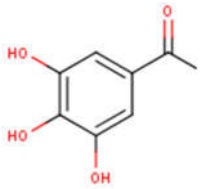
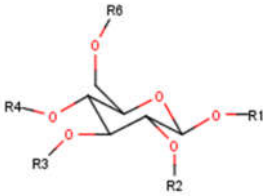


Fig. 1. UHPLC-PDA chromatogram (monitored at 254 nm) of the gallnut dyed silk extract.

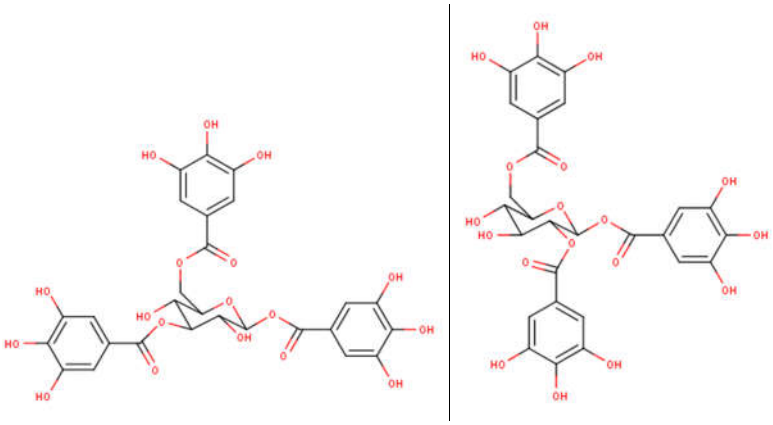




Gallic acid Galloyl group

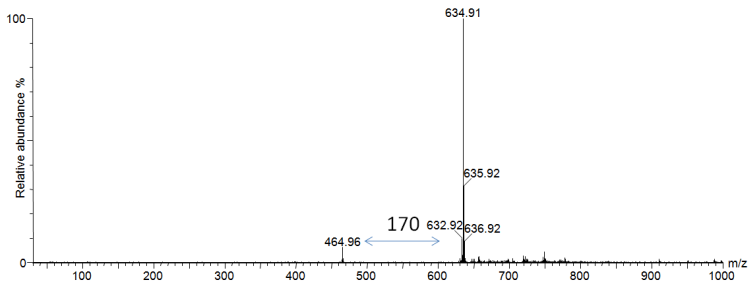


Galloyl esterification, R= H or galloyl group(s).

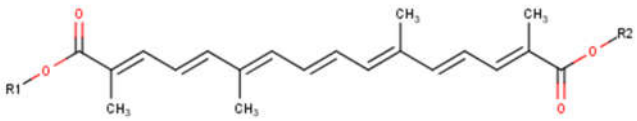


1,3,6-Trigalloyl glucose 1,2,6-Trigalloyl glucose

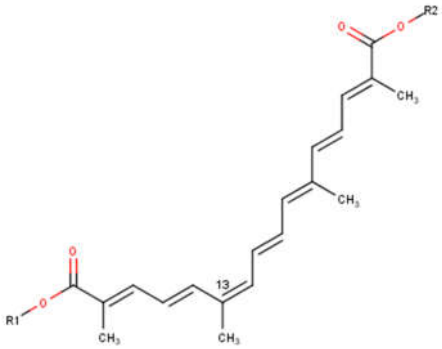
(a)



(b)

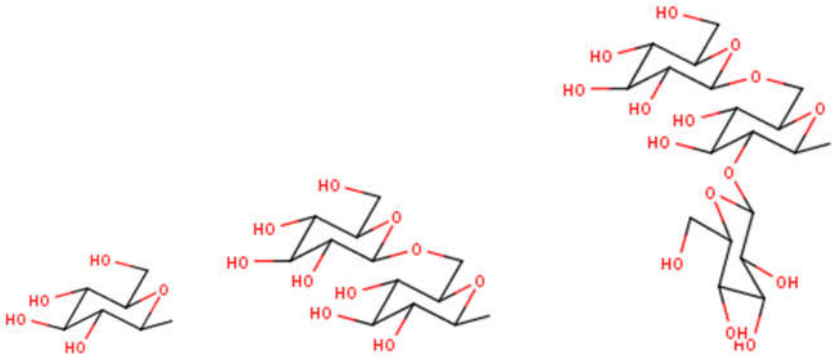


all-*trans*-Crocetin/Crocin



13-c*is*-Crocetin/Crocin

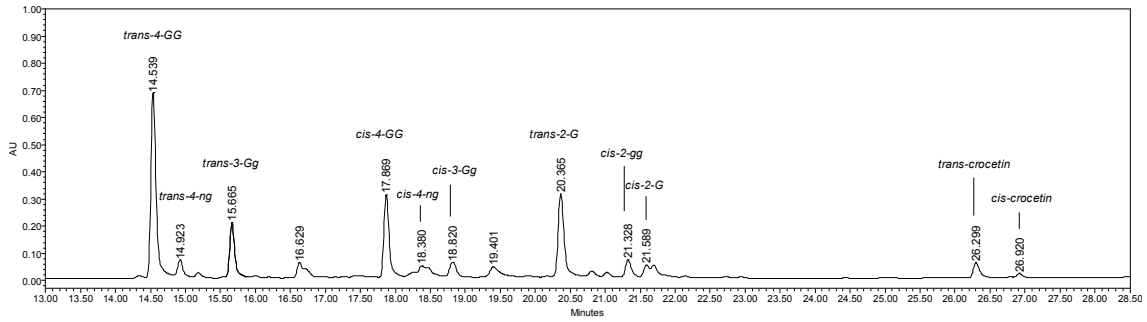
R = H, Glucosyl (g), Gentiobiosyl (G) or Neapolitanosyl (n).



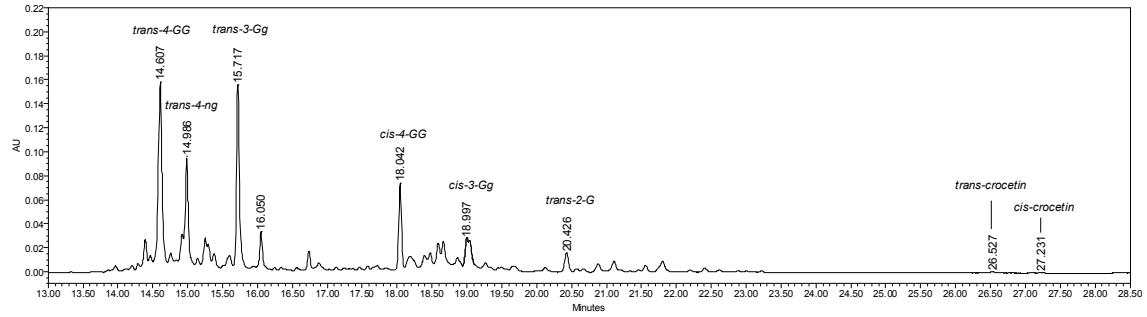
β-D-Glucosyl (g) β-D-Gentiobiosyl (G) β-D-Neapolitanosyl (n)

(c)

Fig. 2. Esterification and isomerisation patterns of the dye components of gallnut and gardenia, and a demonstrative mass spectrum. (a) Examples of the isomerisation and esterification of gallotannins. (b) A negative ion mode ESI mass spectrum of trigalloyl glucose for demonstration. The m/z of the molecular ion is 635 Da. The fragment ion at m/z=465 Da is due to loss of a gallic acid unit (170 Da). (c) The isomerisation and glycosyl esterification of crocetin [25].

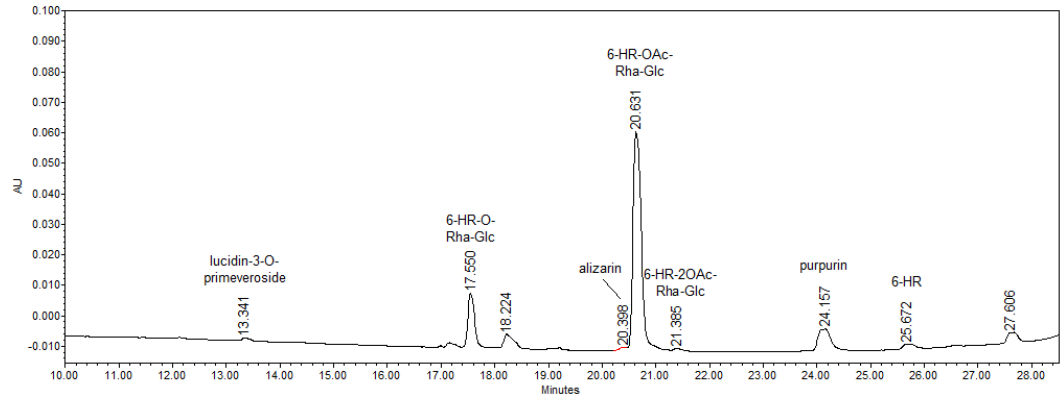


(a)

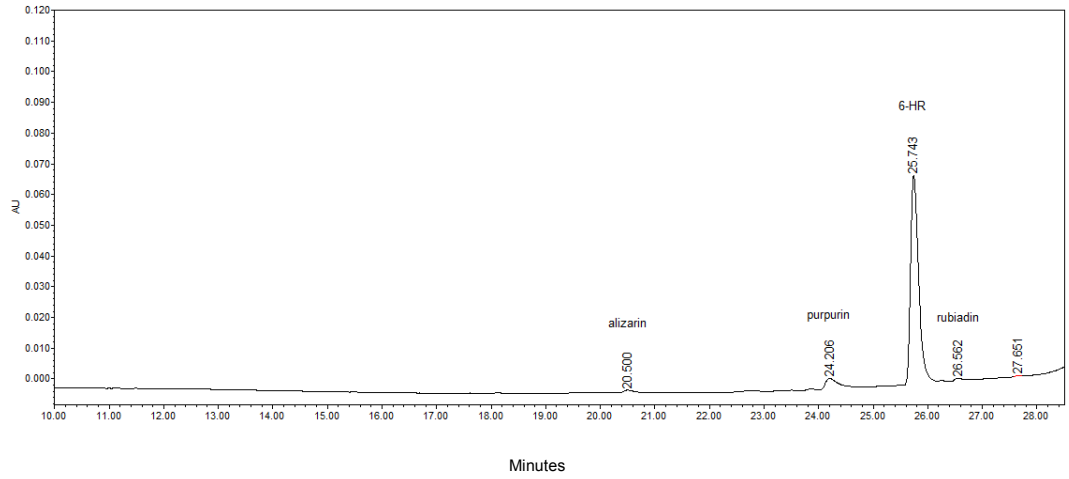


(b)

Fig. 3. UHPLC-PDA chromatograms (monitored at 425 nm) of (a) the gardenia dyed silk extract and (b) a saffron dyed silk extract. g: Glucosyl; G: Gentibiosyl; n: Neapolitanosyl.



(a)



(b)

Fig. 4. UHPLC-PDA chromatograms (monitored at 430 nm) of the munjeet dyed silk sample extracted by (a) DMSO-OA and (b) HCl. 6-HR: 6-hydroxyrubiadin; Glc: glucose; Rha: rhamnose; Ac: Acetyl.

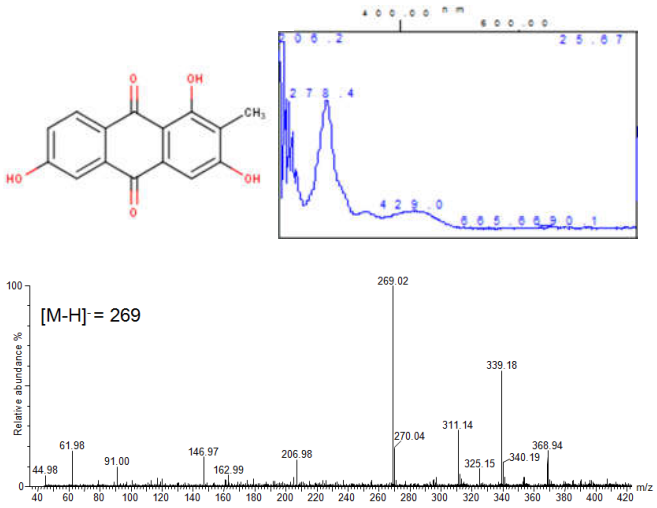


Fig. 5. 6-Hydroxyrubiadin ($C_{15}H_{10}O_5$): its structure formula, UV-Vis spectrum and ESI mass spectrum.

Table 1

Chromatographic, UV-Vis spectral and mass spectral data of the characteristic components of the dyes.

Dye	Retention time (min)	λ_{max} (nm)	$[M - H]^-$ (m/z) [b]	Component identified	Notes
gallnut	3.0	219, 271	169, 125, 283	gallic acid monomer	125: loss of $[M\text{-}H\text{-}CO_2]^-$ (44) 283: possibly ellagic acid anhydride
	6.6	216, 277	483	digalloyl glucose	
	8.2	219, 277	635, 465	trigalloyl glucose	465: loss of a gallic acid unit (170)
	9.5	219, 278	787	tetragalloyl glucose	
	11.8	219, 280	939, 769, 469	pentagalloyl glucose	769: loss of a gallic acid unit (170) 469: doubly charged ion $[M - 2H]^{2-}$
	13.7	219, 278	1091, 302	hexagalloyl glucose	302: probably ellagic acid coeluting
	14.6	216, 277	1243, 621	heptagalloyl glucose	621: doubly charged ion $[M - 2H]^{2-}$
gardenia	14.5	261, 328, 441, 464	975, 813, 651, 327	*trans*-4-GG-crocin[a]	
	14.9	261, 326, 437, 463	975, 651	*trans*-4-ng-crocin	
	15.7	261, 327, 440, 464	813, 651, 489, 327	*trans*-3-Gg-crocin	
	16.6	219, 328, 439, 463	975, 489, 327	crocin equivalent	
	17.9	262, 325,	975, 651	*cis*-4-GG-crocin	

		434, 458			
	18.4	226, 323, 435, 459	975	*cis*-4-ng-crocin	
	18.8	261, 325, 434, 459	813, 651	*cis*-3-Gg-crocin	
	19.4	219, 328, 437, 462	813	crocin equivalent	
	20.4	258, 323, 434, 458	651, 327	*trans*-2-G-crocin	
	21.4	259, 320, 428, 454	651	*cis*-2-gg-crocin	
	21.6	259, 323, 428, 453	651, 489, 327	*cis*-2-G-crocin	
	26.3	255, 318, 427, 452	327	*trans*-crocetin	
	27.0	257, 318, 421, 445	327	*cis*-crocetin	
munjeet	13.3	265, 404	563	lucidin-3-O-primeveroside	present in the DMSO-OA extract
	17.6	275, 416	577	6-hydroxyrubiadin-O-Rha-Glc	DMSO-OA
	18.2	275, 416	619	6-hydroxyrubiadin-O-Rha-Glc (an isomer of a small amount)	DMSO-OA
	20.5	249, 280, 431	below LOD	alizarin	DMSO-OA, HCl
	20.6	275, 416	619	6-hydroxyrubiadin-OAc-Rha-Glc	DMSO-OA
	21.4	277, 417	below LOD	6-hydroxyrubiadin isomer	DMSO-OA
	24.2	256, 295, 480	255	purpurin	DMSO-OA, HCl
	25.7	278, 344, 429	269	6-hydroxyrubiadin	DMSO-OA, HCl (much higher)
	26.5	204, 244, 278, 408	253	rubiadin	HCl
turmeric	22.8	263, 428	367	curcumin	
	23.5	250, 424	337	desmethoxycurcumin	
	24.3	248, 418	307	bisdesmethoxycurcumin	

pagoda bud	13.6	256, 354	609	rutin
	15.1	255, 352	623	isorhamnetin-3-rutinoside
	15.4	253, 366	593	kaempferol-3-rutinoside
	18.4	255, 371	301	quercetin
	20.6	253, 371	315	isorhamnetin
	21.0	265, 366	285	kaempferol
indigo	6.4	242, 302, 419	146	isatin
	21.7	240, 286, 334, 614	261	indigotin
	23.9	210, 238, 289, 364, 544	261	indirubin
acorn cup	7.5	220, 251, 374	-	ellagic acid equivalent
	9.4	203, 214, 278	see gallnut	pentagalloyl glucose
	11.6	218, 278	see gallnut	hexagalloyl glucose
	13.6	253, 367	*301*	ellagic acid
Chinese cork tree	7.8	227, 264, 347, 423	*337 (positive mode)*	berberine
smoketree	16.0	209, 364	*285*	fisetin
	17.2	203, 257, 398	*269*	sulfuretin
sappanwood	7.4	203, 287	*285*	brasilin
	7.6	207, 253, 287	-	Type A component
	8.1	207, 253, 284	-	Type A component
	8.3	202, 294, 451	*283*	brasilein
	8.4	211, 253, 291	-	Type A component
	14.5	259, 306,	-	Type C component

		340		
safflower	15.3	204, 268	*582.5*	Ct1
	16.2	210, 287	*582.5*	Ct2
	16.8	291	*582.5*	Ct3
	16.9	210, 295	*582.5*	Ct4
	17.5	206, 293	-	another Ct component
	23.4	244, 374, 520	*909.5*	carthamin
gromwell	17.3	277, 516	-	shikonin equivalent
	22.9	277, 516	*287*	shikonin

[a] n: neapolitanoside; G: gentibioside; g: glucoside

[b] MS data of the main components of acorn cup, Chinese cork tree, smoketree, sappanwood, safflower and gromwell (in *italics*) was consulted from published articles where ESI-MS, the same method as used in this research, was applied [9, 27-31].

Electronic Supplementary Material

Appendix A. Dye recipes

Table A.1

Dye recipes for reference samples.

Dye plant	Amount (g)	Main additives	Dyeing method	General colours achieved
safflower	30	citric acid and alkali	acidic dyeing	red
sappanwood	1	alum	post-mordanting	brown
munjeet	1	alum	pre-mordanting	red
gromwell	1. 5	alum	pre-mordanting	purple
smoketree	0. 5	alum	post-mordanting	yellow
Chinese cork tree	0. 5	——	direct dyeing	yellow
turmeric	5	——	direct dyeing	yellow
pagoda bud	1	alum	post-mordanting	yellow
gardenia	1	——	direct dyeing	yellow
indigo	1	——	vat dyeing	blue
gallnut	0. 3	ferrous sulphate	post-mordanting	black
acorn cup	0. 5	ferrous sulphate	post-mordanting	black

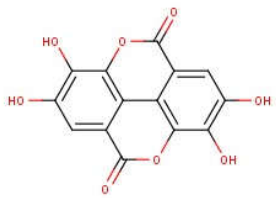
Souring. For each dye recipe 20×20 cm (1.3 g) of silk was used. Before dyeing, silk was scoured with soda (pH=9) and simmered for 15 minutes to remove impurities.

Extracting dye components from dyes. Dyes were cut into small pieces or ground to powders, soaked in 150-200 ml deionised water for 20 minutes, brought to a boil and simmered at 90 ℃ for 30 minutes. The dye bath was obtained by filtering the solution through a piece of wet cotton. This procedure of adding water, heating and filtering was repeated once. Altogether 250-300 ml of dye bath was obtained. The dyeing processes of pagoda buds and gromwell were slightly different. Pagoda buds were fried with mild heat before extracting dyes according to historical records, e.g. a dye recipe in *Duoneng bishi*. For the extraction of gromwell dyes, the water solution was adjusted to pH 10 to help the dissolution of dyes and then adjusted back to pH 5 during dyeing. Extraction and dyeing were carried out at 50-60℃ for 40 minutes to prevent possible decomposition of the dye components of gromwell.

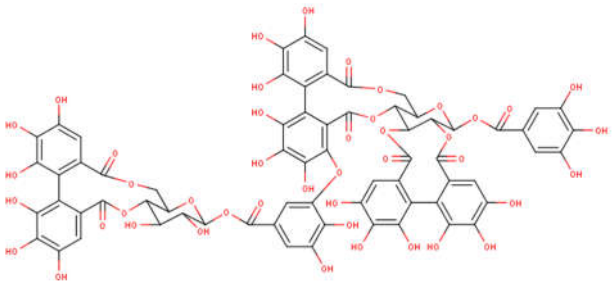
Dyeing. A piece of silk was wetted, soaked in the dye bath at 40°C, heated slowly to 60-70°C and immersed for an hour with regular stirring. The fabric was then rinsed with deionised water and left to dry, protected from light. For dyeing with safflower an acidic dyeing methodology was used, and reduction dyeing was used for indigo, because of the particular chemical properties of the dyes.

Mordanting. Mordants used included alum (2 g/L) and ferrous sulphate (0. 05 g/L). The mordant was dissolved in deionised water (for pre-mordanting) or in the dye bath (for post-mordanting) and the mordant bath was heated to 40 °C. The wet fabric was soaked into the bath and the bath continued to be heated slowly to 60-70 °C, kept for 15-30 minutes and then allowed to cool. Surplus mordant on the fabric was rinsed away. Fabrics were dried in air. Alum pre-mordanted fabrics were kept wet till dyeing.

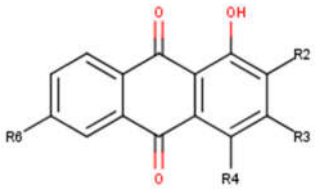
Appendix B. Chemical structures



Ellagic acid

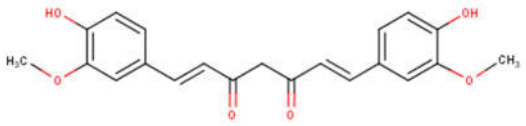


An example of ellagitannin (Sanguiin H-10) [1]

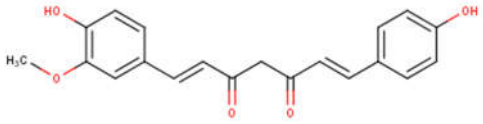


[2]

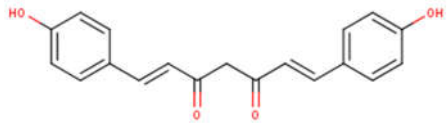
Common name	R2	R3	R4	R6
Alizarin	OH	H	H	H
6-Hydroxyrubiadin	CH3	OH	H	OH
Lucidin	OH	COOH	OH	H
Munjistin	COOH	OH	H	H
Pseudopurpurin	OH	H	OH	H
Purpurin	CH3	OH	H	H
Rubiadin	CH2OH	OH	H	H



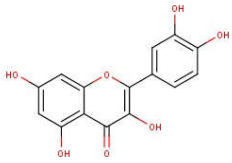
Curcumin



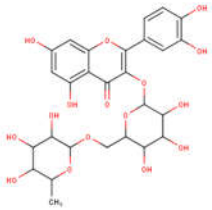
Desmethoxycurcumin



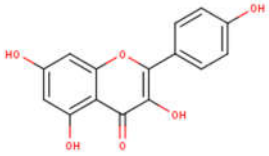
Bisdesmethoxycurcumin



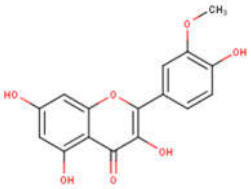
Quercetin



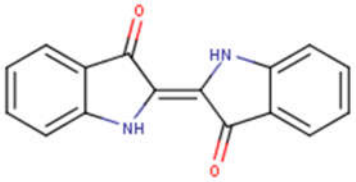
Rutin



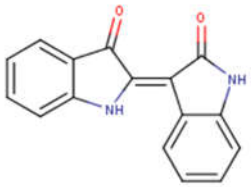
Kaempferol



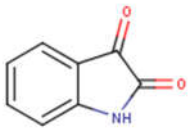
Isorhamnetin



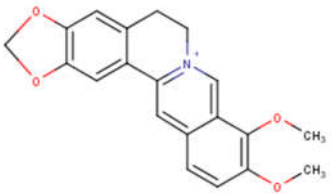
Indigotin



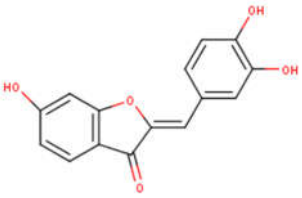
Indirubin



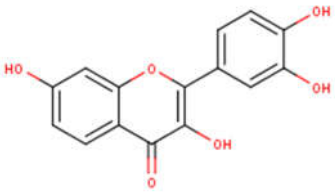
Isatin



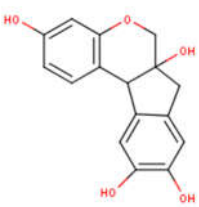
Berberine



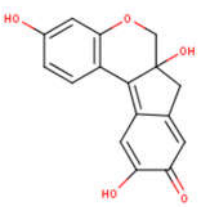
Sulfuretin



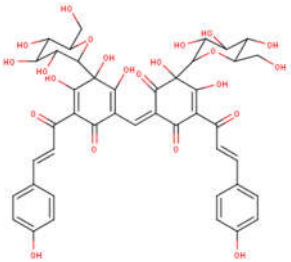
Fisetin



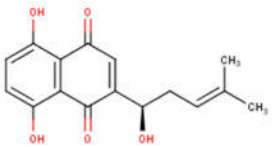
Brasilin



Brasilein



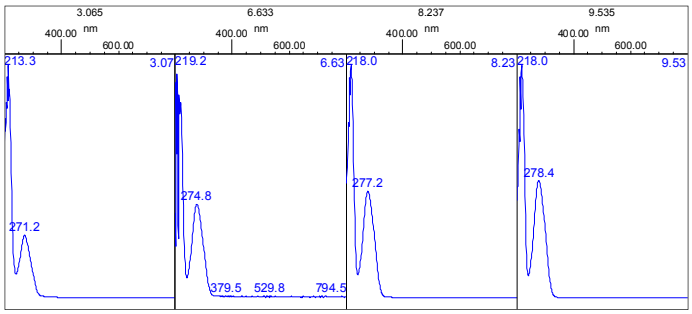
Carthamin

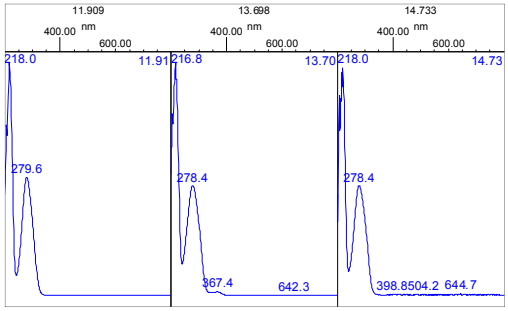


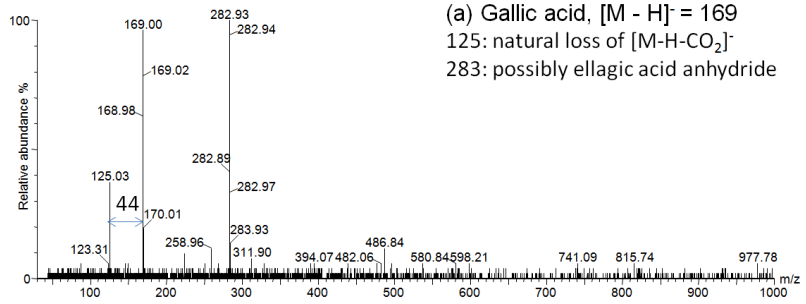
Shikonin

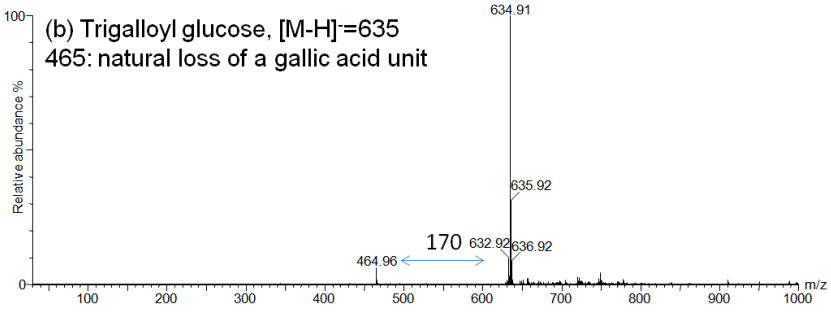
Fig. B.1. Chemical structures of dye components referred to in this article.

Appendix C. Chromatograms, UV-Vis spectra and mass spectra of the dyed samples









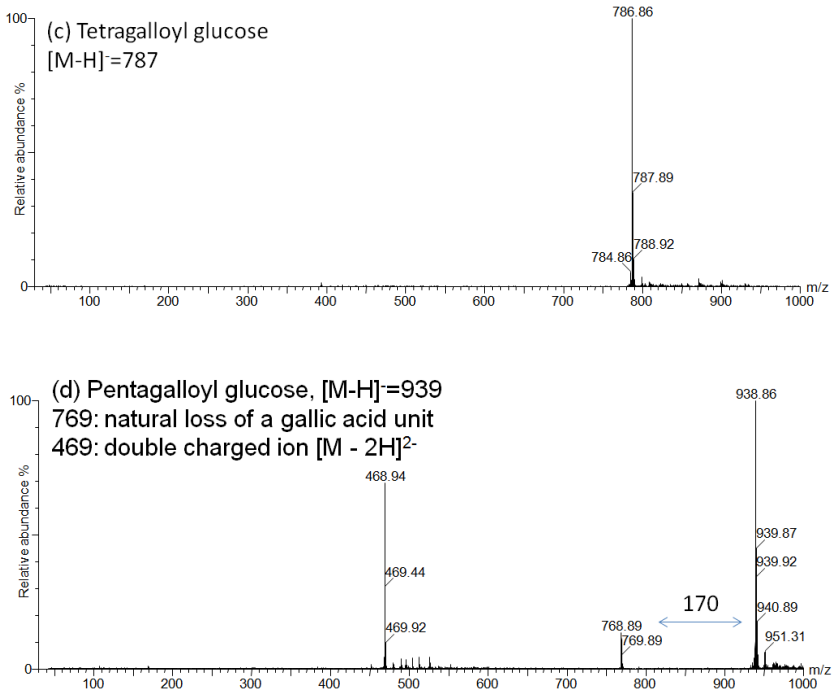
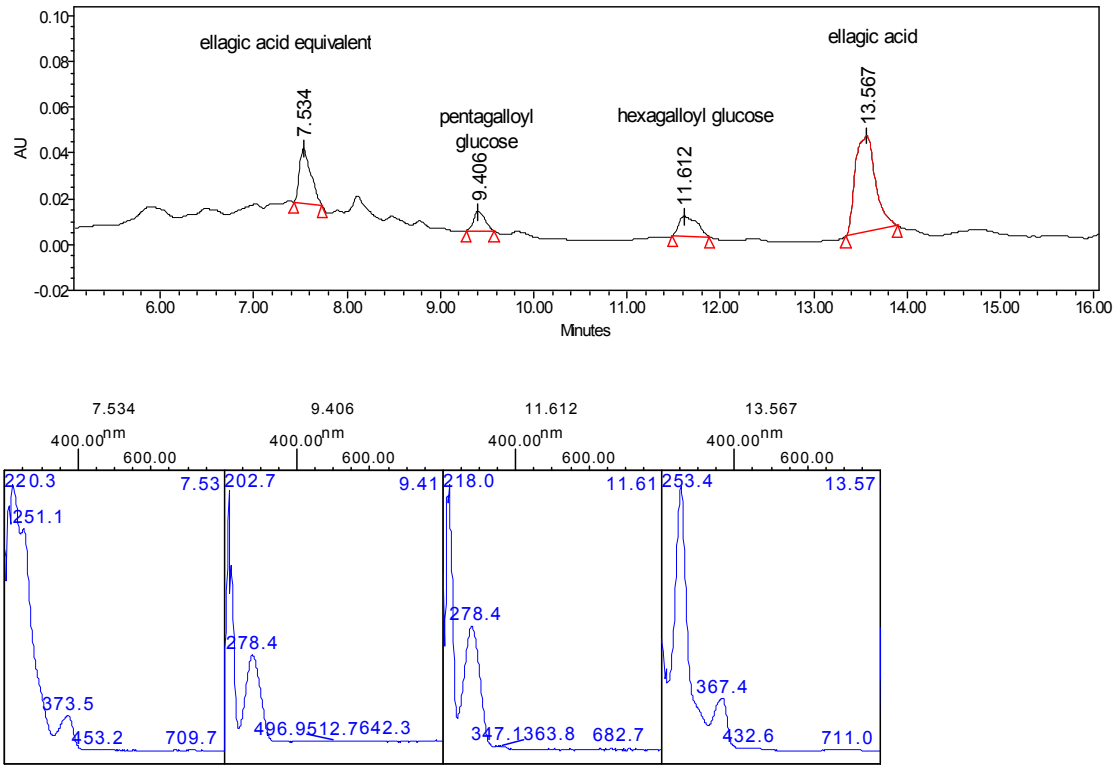


Fig. C.1. UV-Vis spectra and mass spectra of the main constituents of the gallnut dyed silk extract.



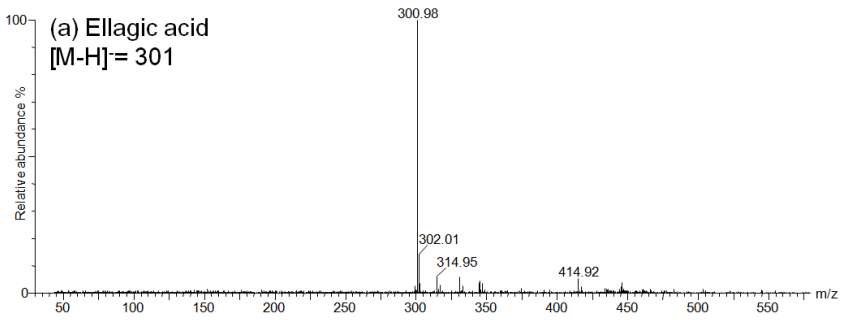
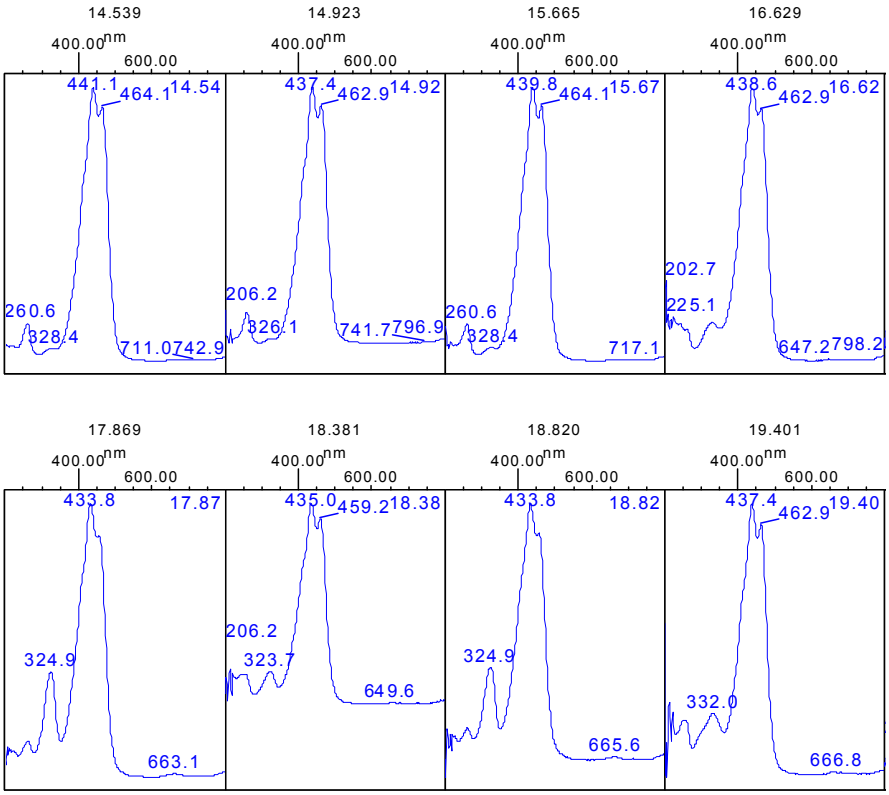
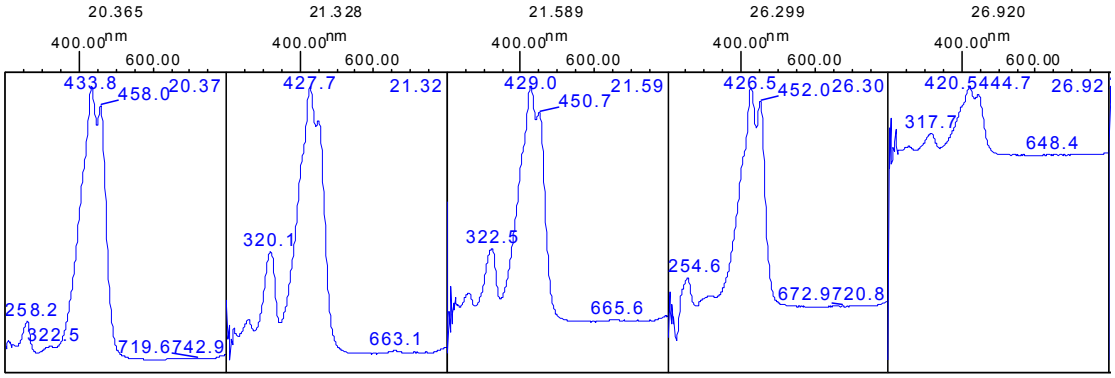
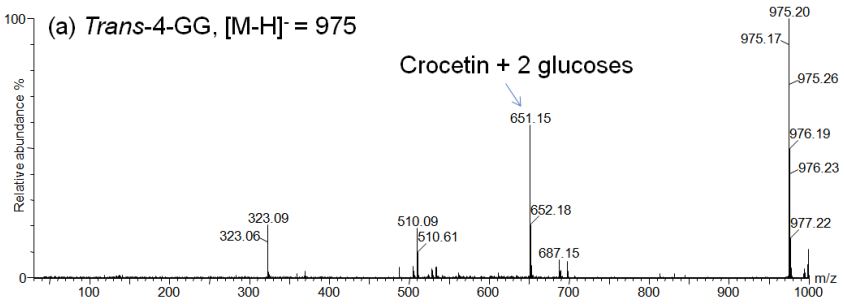
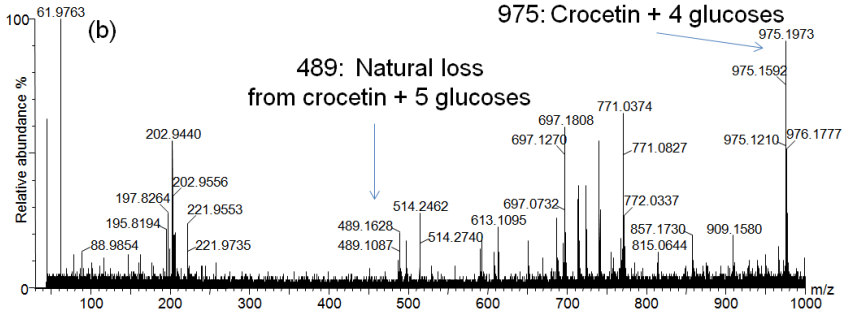


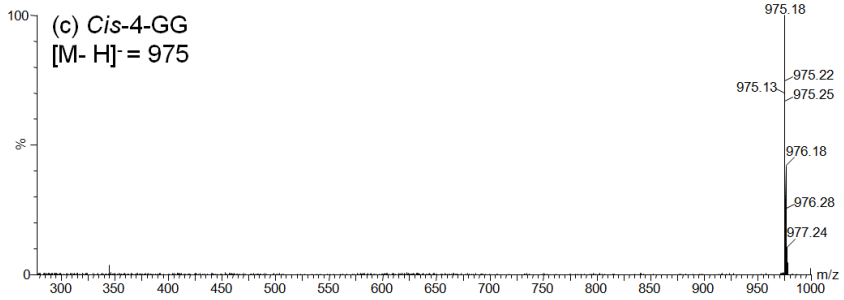
Fig. C.2. UHPLC-PDA chromatogram (monitored at 280 nm) of the acorn cup dyed silk extract, and UV-Vis spectra and a mass spectrum of its main constituents.

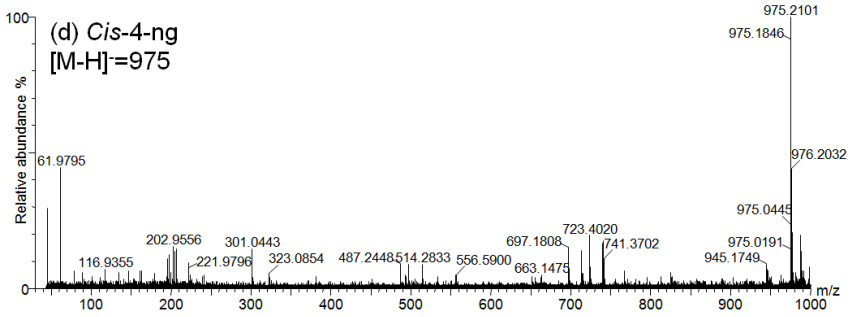


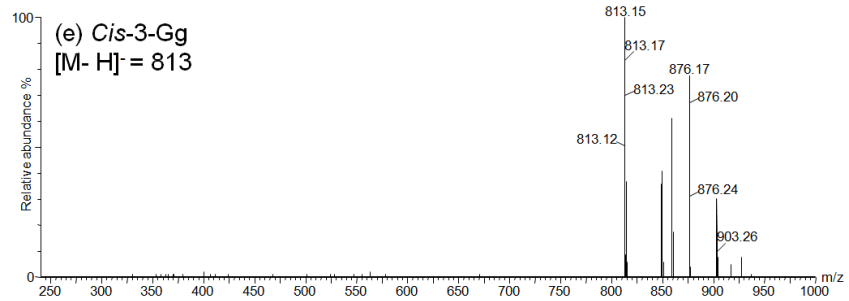

20.365
21.328
21.589
26.299
26.920
400.00nm
600.00
433.8
458.0
20.37
427.7
21.32
429.0
450.7
21.59
426.5
452.0
26.30
420.5
444.7
26.92
317.7
648.4
320.1
322.5
254.6
258.2
322.5
719.6
742.9
663.1
665.6
672.9
720.8

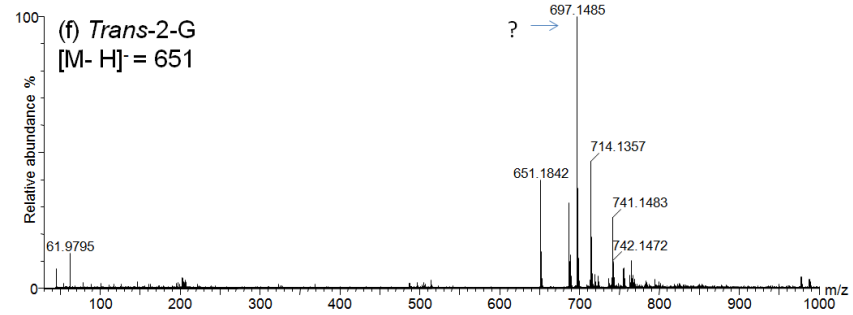

(a) Trans-4-GG, [M-H]- = 975
Crocetin + 2 glucoses
Relative abundance %
975.20
975.17
975.26
651.15
976.19
976.23
652.18
323.09
323.06
510.09
510.61
687.15
977.22
m/z

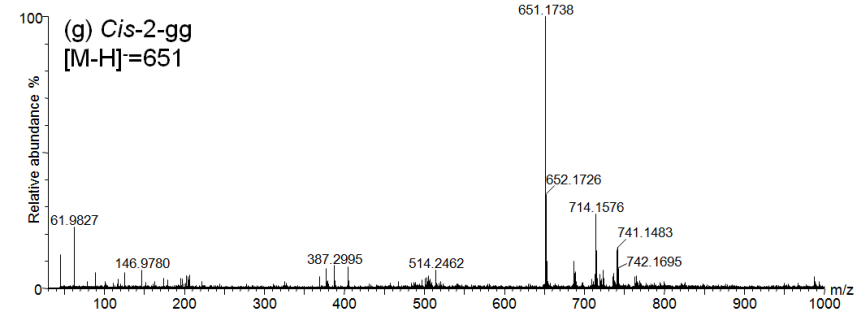

(b)
975: Crocetin + 4 glucoses
489: Natural loss
from crocetin + 5 glucoses
Relative abundance %
61.9763
975.1973
975.1592
771.0374
697.1808
202.9440
697.1270
771.0827
975.1210
976.1777
202.9556
197.8264
221.9553
514.2462
697.0732
772.0337
195.8194
489.1628
613.1095
857.1730
909.1580
88.9854
221.9735
489.1087
514.2740
815.0644
m/z

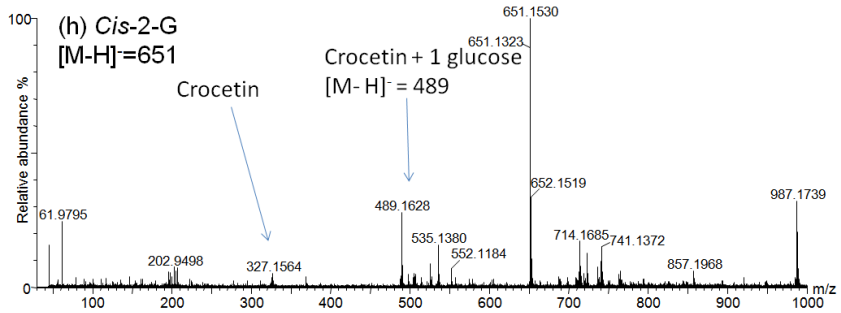

(c) Cis-4-GG
[M- H]- = 975
%
975.18
975.22
975.13
975.25
976.18
976.28
977.24
m/z


(d) Cis-4-ng
[M-H]-=975
Relative abundance %
m/z
975.2101
975.1846
976.2032
975.0445
975.0191
945.1749
61.9795
116.9355
202.9556
221.9796
301.0443
323.0854
487.2448
514.2833
556.5900
663.1475
697.1808
723.4020
741.3702


(e) Cis-3-Gg
[M- H]- = 813
Relative abundance %
m/z
813.15
813.17
876.17
813.23
876.20
813.12
876.24
903.26


(f) Trans-2-G
[M- H]- = 651
Relative abundance %
m/z
?
697.1485
714.1357
651.1842
741.1483
742.1472
61.9795


(g) Cis-2-gg
[M-H]-=651
Relative abundance %
m/z
651.1738
652.1726
714.1576
741.1483
742.1695
61.9827
146.9780
387.2995
514.2462


(h) Cis-2-G
[M-H]-=651
Crocetin
Crocetin + 1 glucose
[M- H]- = 489
Relative abundance %
m/z
651.1530
651.1323
652.1519
489.1628
987.1739
61.9795
535.1380
552.1184
714.1685
741.1372
202.9498
327.1564
857.1968

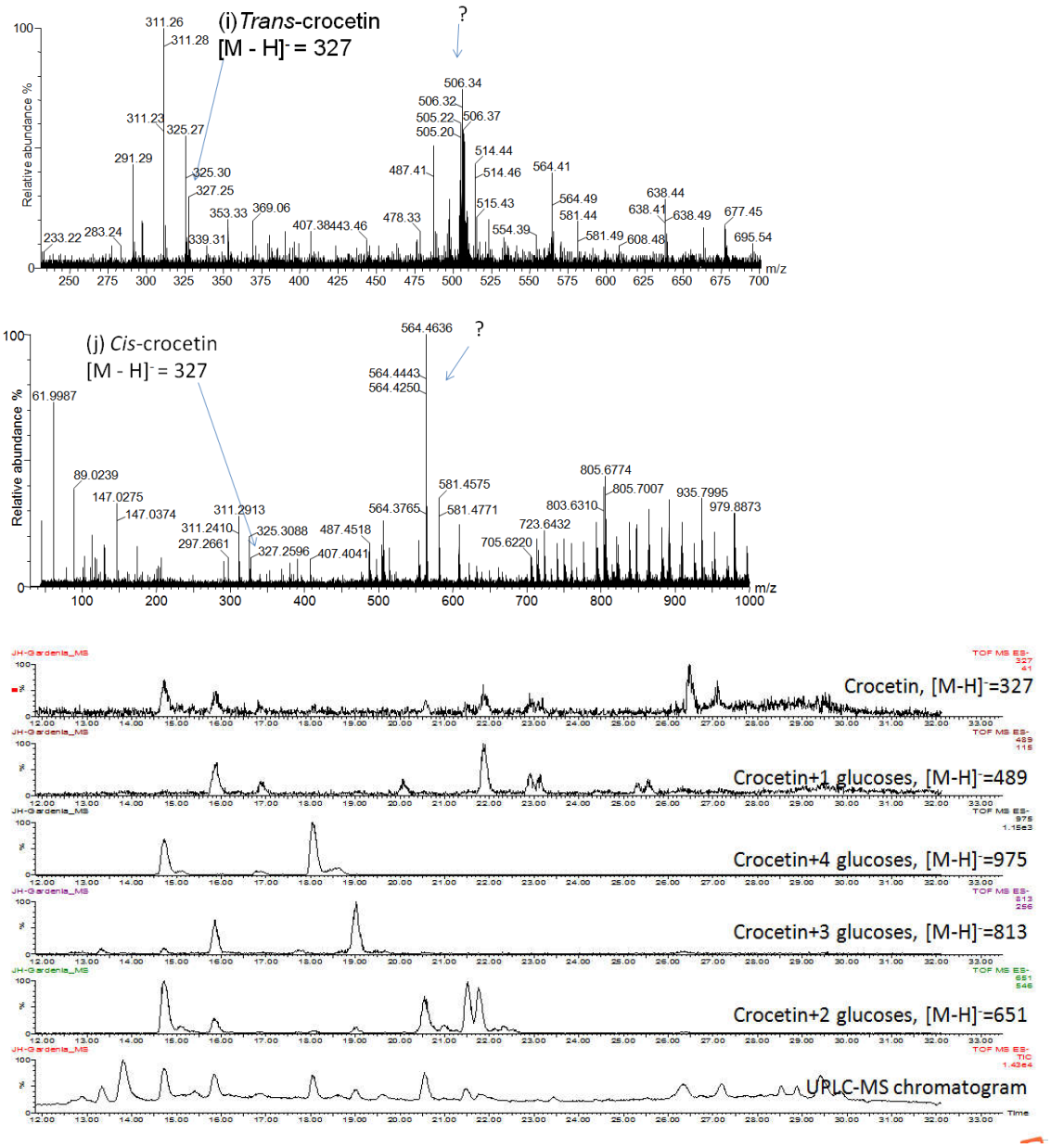


Fig. C.3. UV-Vis spectra and mass spectra of the main constituents of the gardenia dyed silk extract. UHPLC-MS chromatograms of the gardenia dyed silk extract.

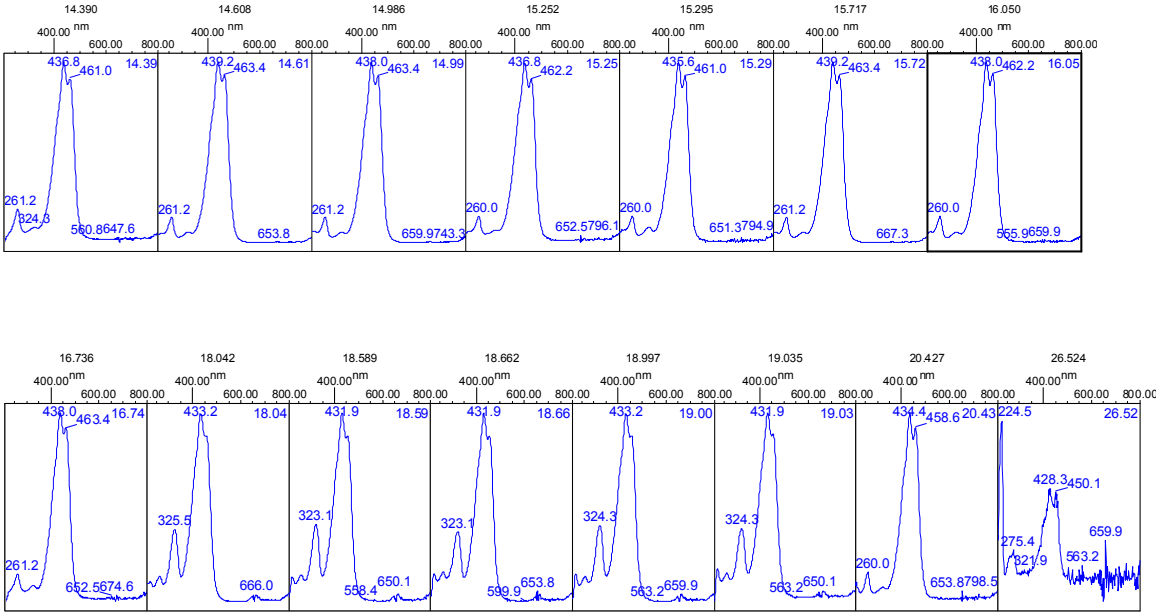
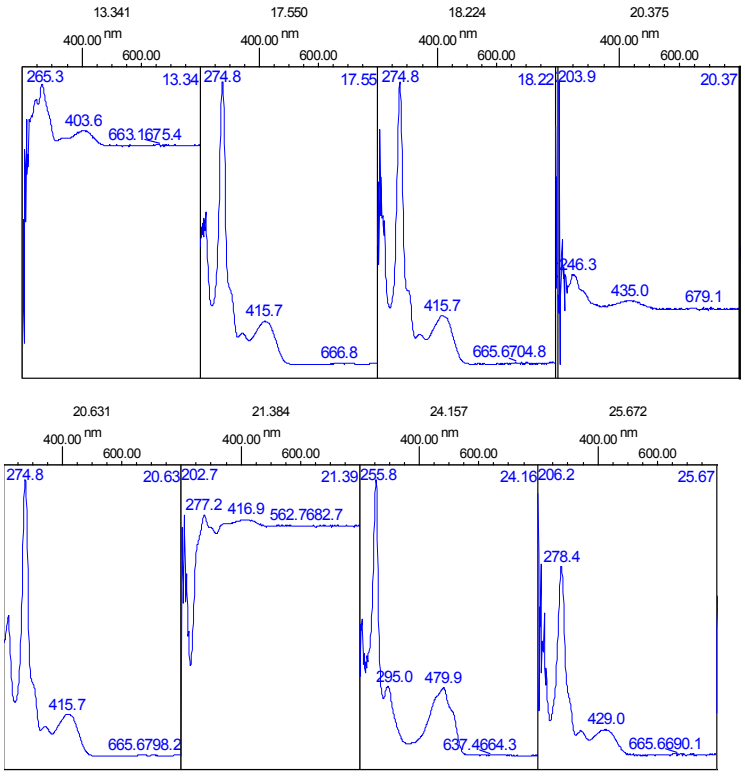
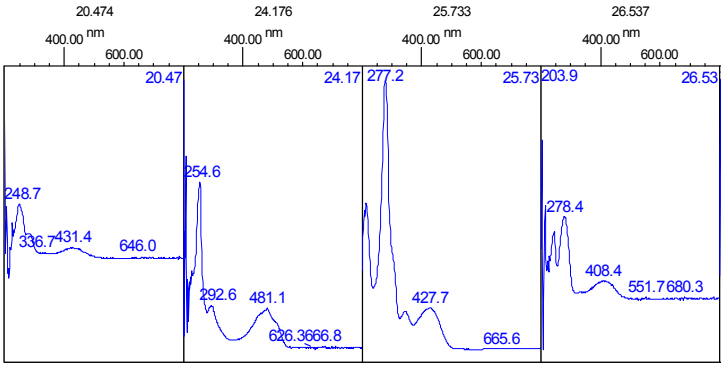


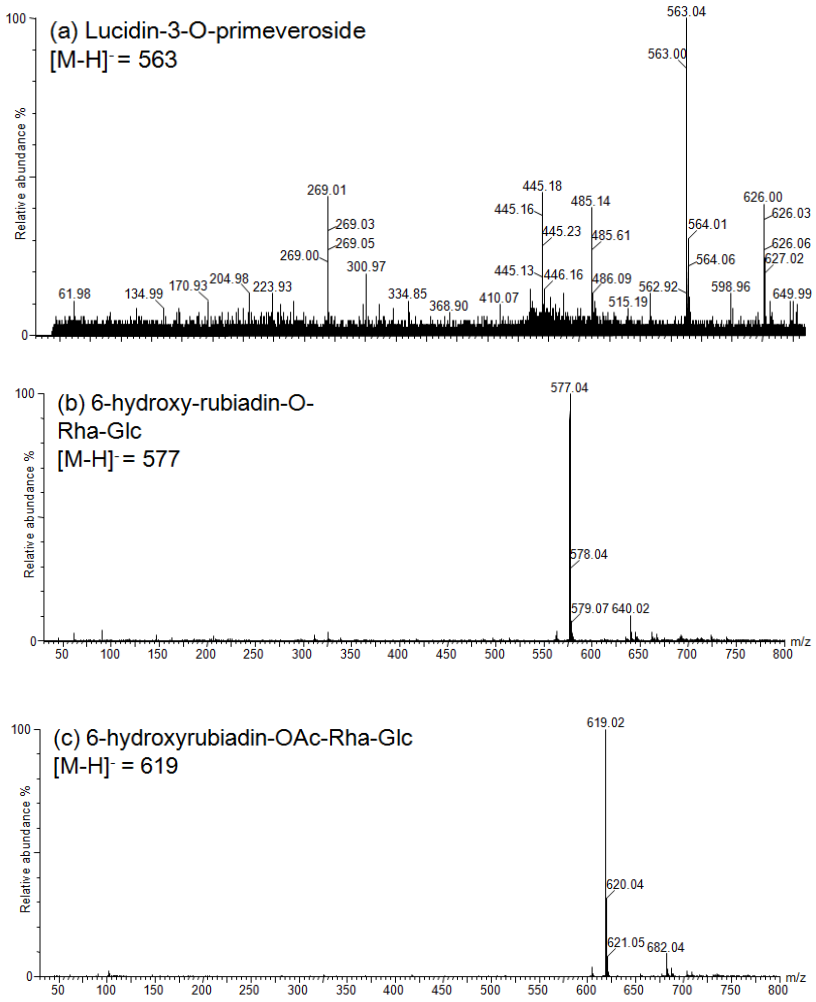
Fig. C.4. UV-Vis spectra of the main constituents of the saffron dyed silk extract.

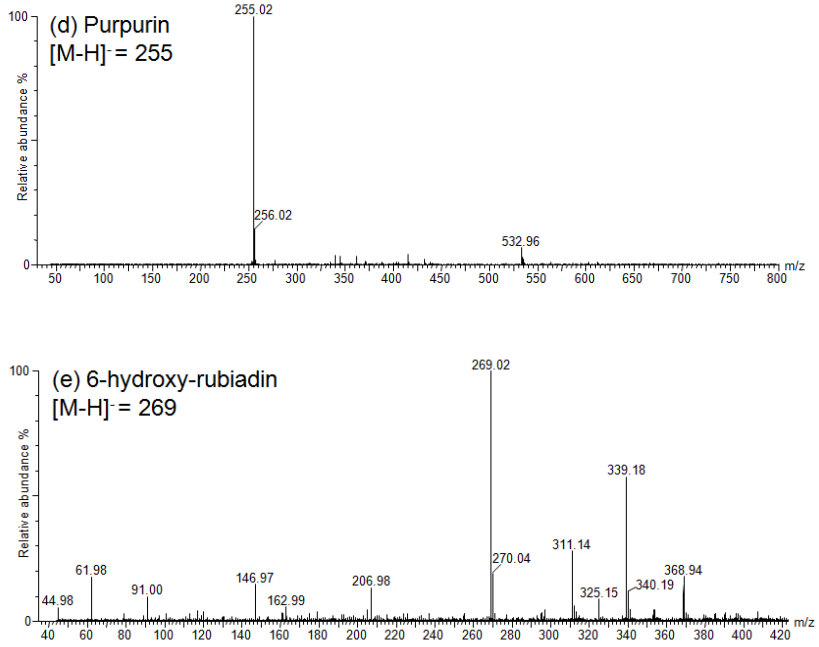


(A)

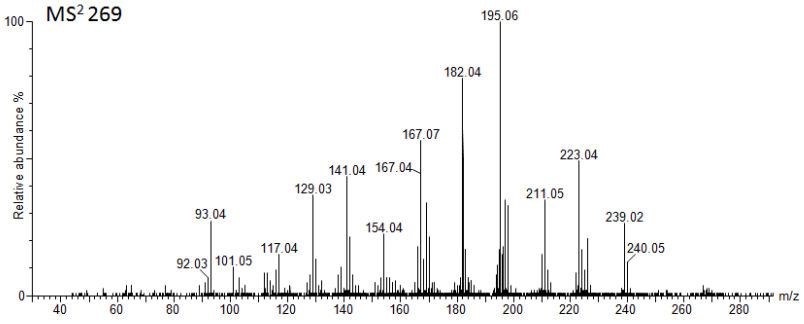

20.474
24.176
25.733
26.537
400.00 nm
600.00
20.47
24.17
277.2
25.73
203.9
26.53
248.7
254.6
336.7
431.4
646.0
292.6
481.1
626.3666.8
427.7
665.6
278.4
408.4
551.7680.3

(B)


(a) Lucidin-3-O-primeveroside
[M-H]- = 563
Relative abundance %
563.04
563.00
269.01
269.03
269.05
269.00
300.97
445.18
445.16
485.14
445.23
485.61
626.00
626.03
564.01
626.06
627.02
564.06
445.13
446.16
486.09
562.92
598.96
649.99
515.19
61.98
134.99
170.93
204.98
223.93
334.85
368.90
410.07
(b) 6-hydroxy-rubiadin-O-Rha-Glc
[M-H]- = 577
577.04
578.04
579.07
640.02
m/z
(c) 6-hydroxyrubiadin-OAc-Rha-Glc
[M-H]- = 619
619.02
620.04
621.05
682.04



(C)



(D)

Fig. C.5. UV-Vis spectra of the main constituents of the munjeet dyed silk sample extract by (A) DMSO-OA and (B) HCl solvent. Mass spectra of the main constituents of the munjeet dyed silk sample extracts (C). MS/MS spectrum of 6-hydroxyrubiadin (D).

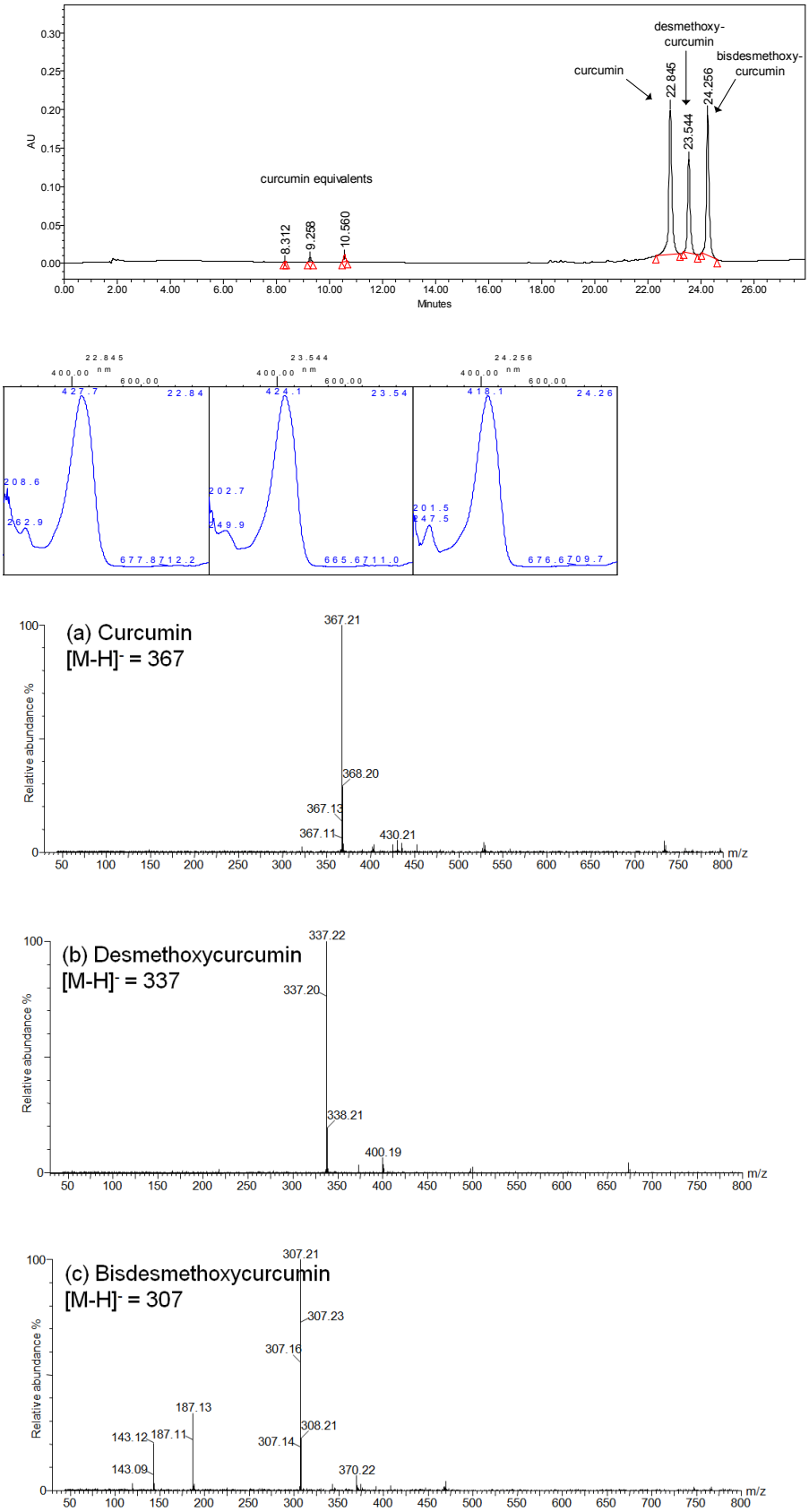
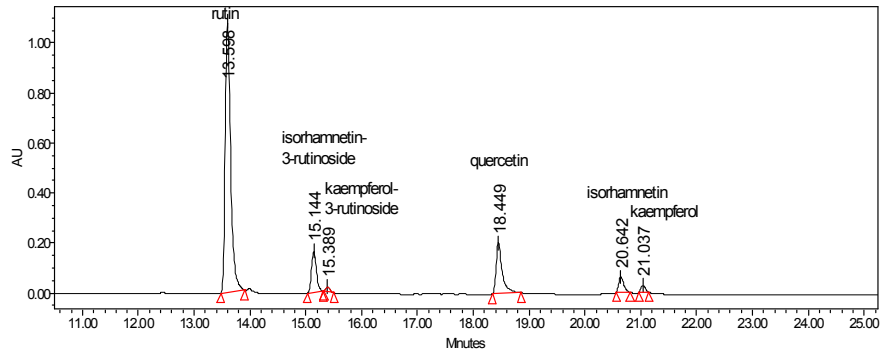
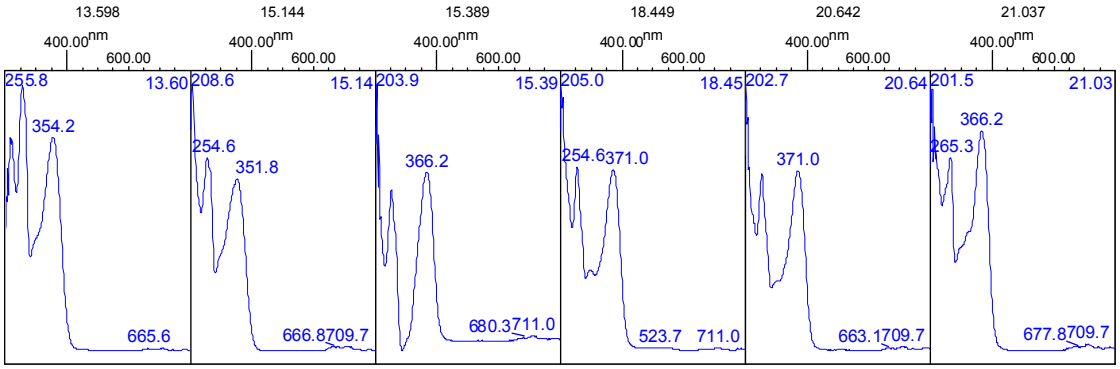
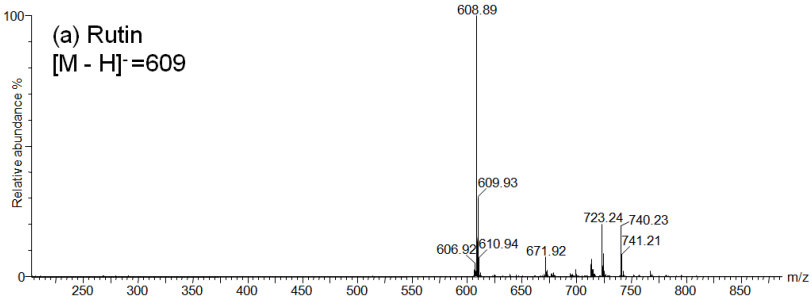
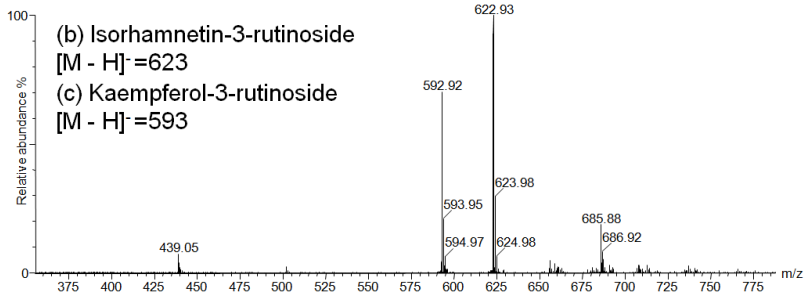


Fig. C.6. UHPLC-PDA chromatogram (monitored at 425 nm) of the turmeric dyed silk extract, and UV-Vis spectra and mass spectra of its main constituents.


rutin
13.598
isorhamnetin-
3-rutinoside
kaempferol-
3-rutinoside
15.144
15.389
quercetin
18.449
isorhamnetin
kaempferol
20.642
21.037
AU
Mnutes


13.598
15.144
15.389
18.449
20.642
21.037
400.00nm
600.00
255.8
354.2
665.6
208.6
254.6
351.8
666.8709.7
203.9
366.2
680.3711.0
205.0
254.6371.0
523.7 711.0
202.7
371.0
663.1709.7
201.5
366.2
265.3
677.8709.7


(a) Rutin
[M - H]- =609
Relative abundance %
608.89
609.93
606.92
610.94
671.92
723.24
740.23
741.21
m/z


(b) Isorhamnetin-3-rutinoside
[M - H]- =623
(c) Kaempferol-3-rutinoside
[M - H]- =593
Relative abundance %
622.93
592.92
623.98
593.95
685.88
686.92
439.05
594.97
624.98
m/z

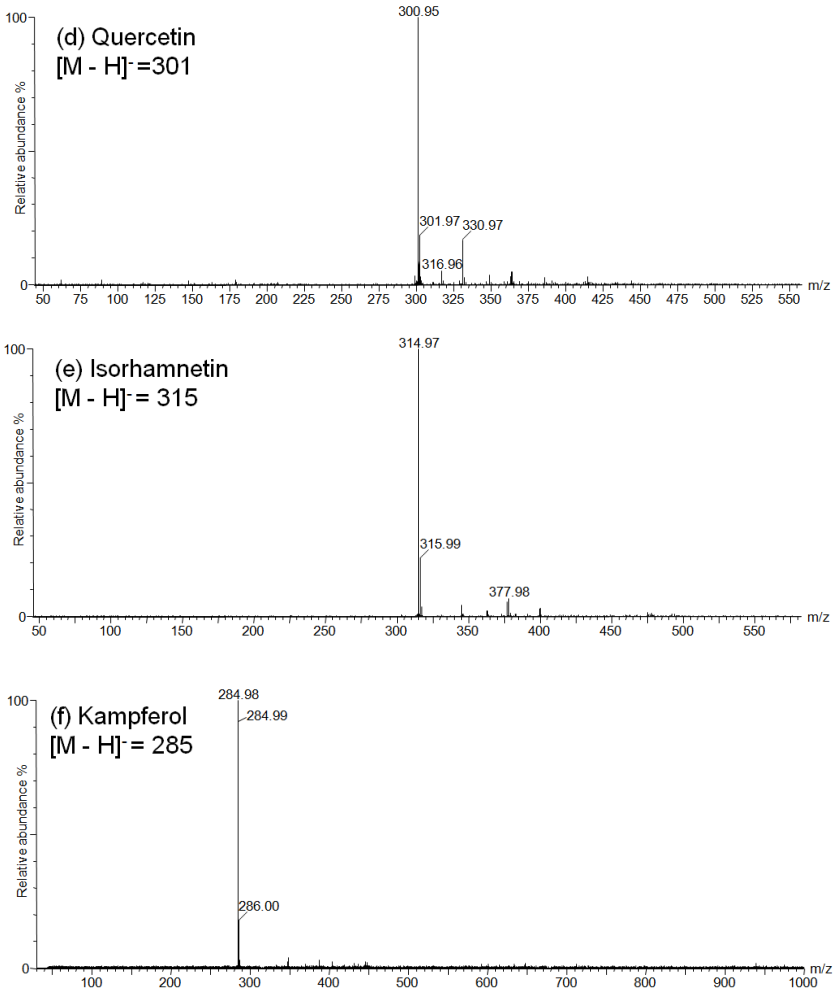
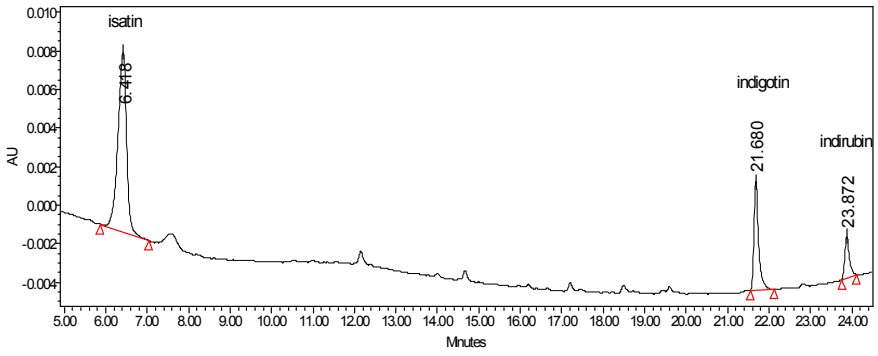


Fig. C.7. UHPLC-PDA chromatogram (monitored at 350 nm) of the pagoda bud dyed silk extract, and UV-Vis spectra and mass spectra of its main constituents.



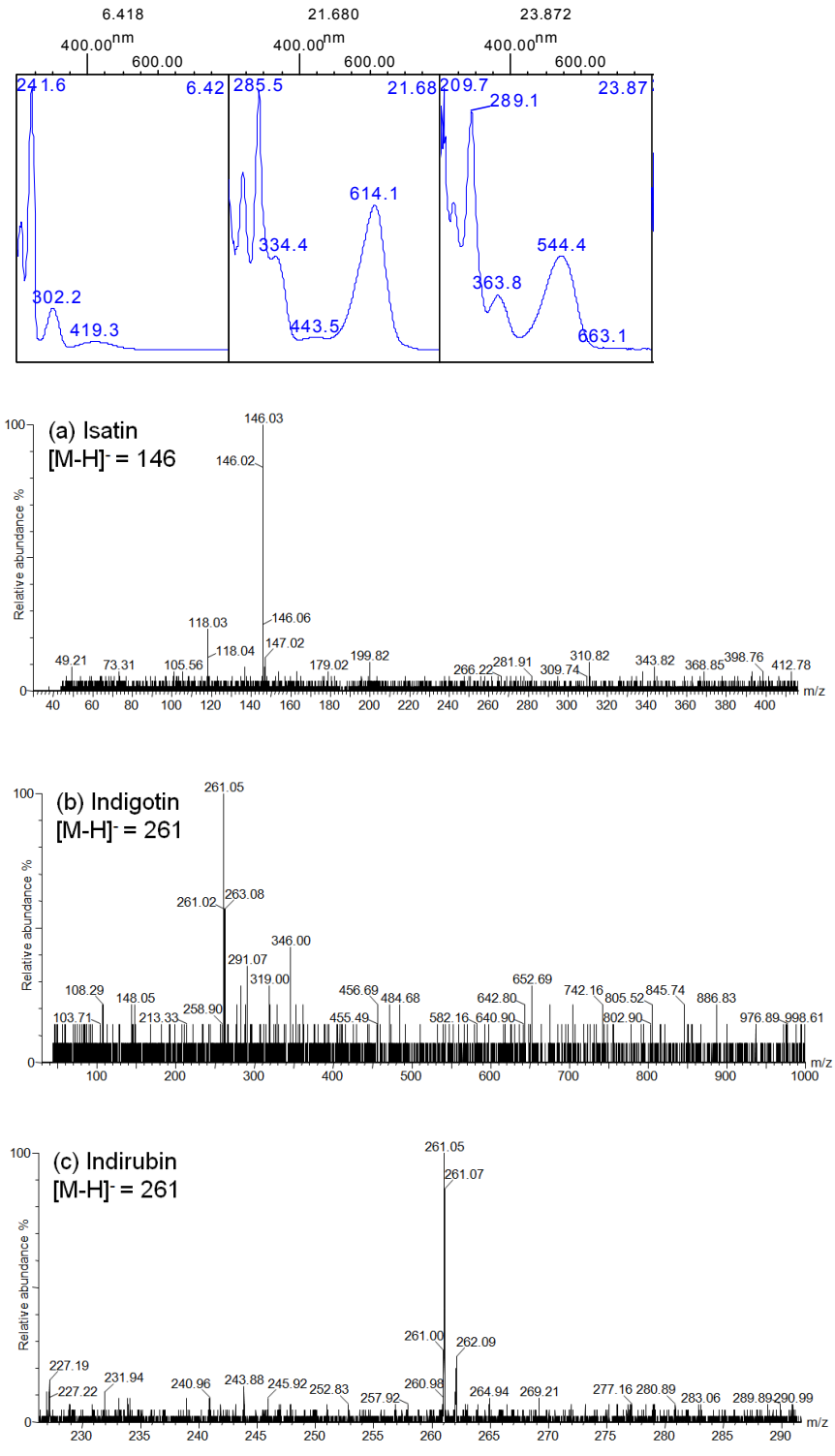


Fig. C.8. UHPLC-PDA chromatogram (monitored at 425 nm) of the indigo dyed silk extract, and UV-Vis spectra and mass spectra of its main constituents.

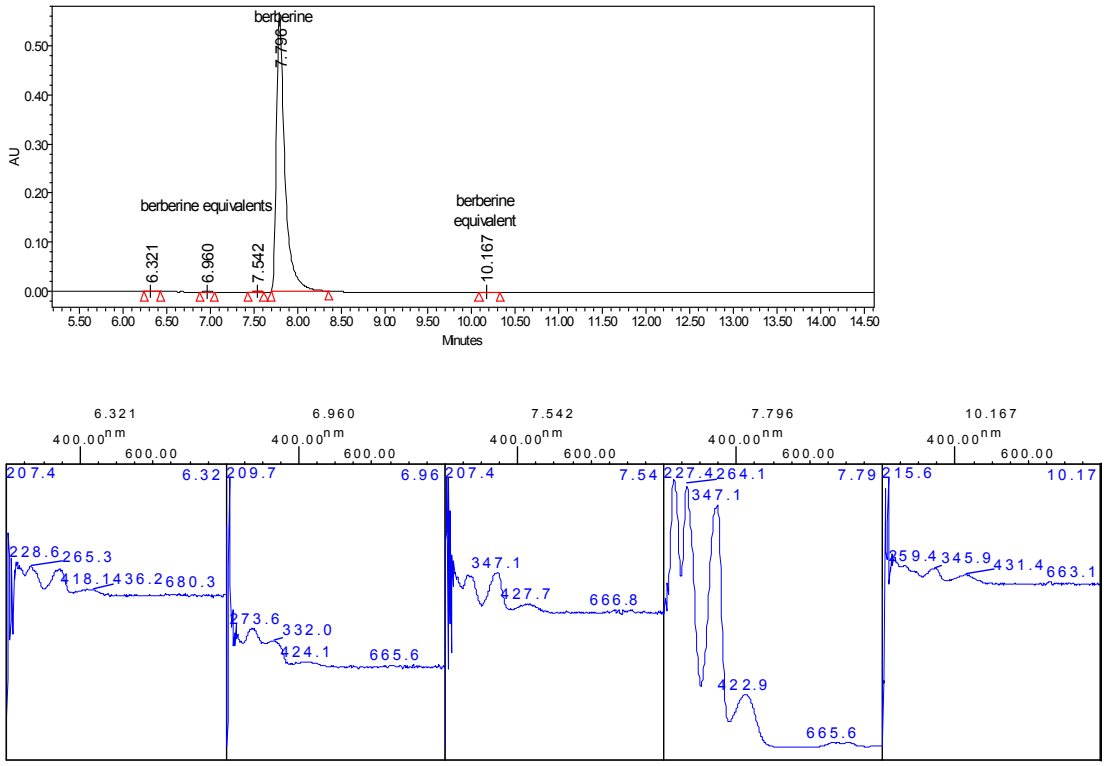


Fig. C.9. UHPLC-PDA chromatogram of the Chinese cork tree dyed silk extract (monitored at 350 nm) and UV-Vis spectra of its main constituents.

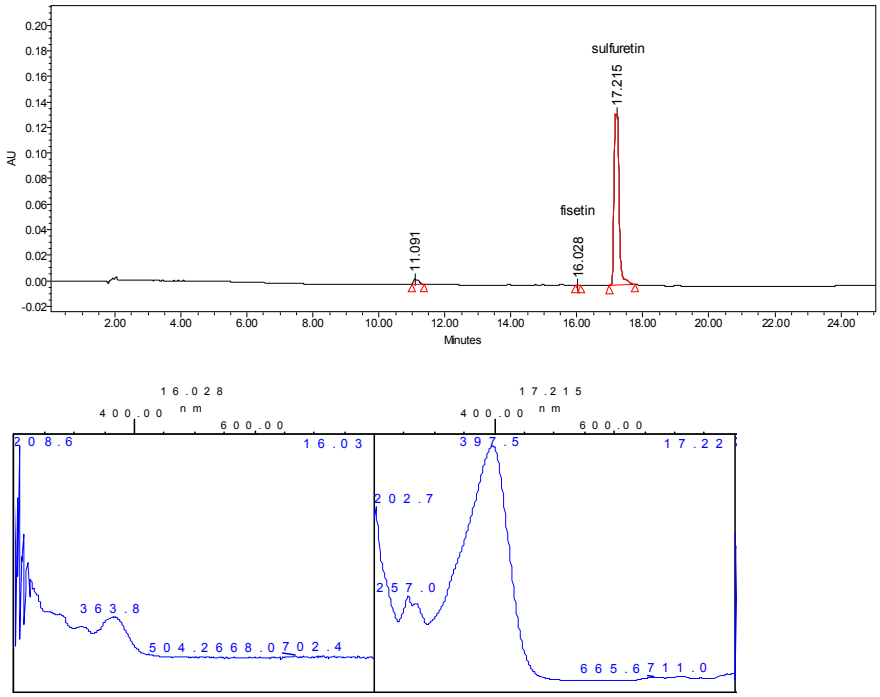
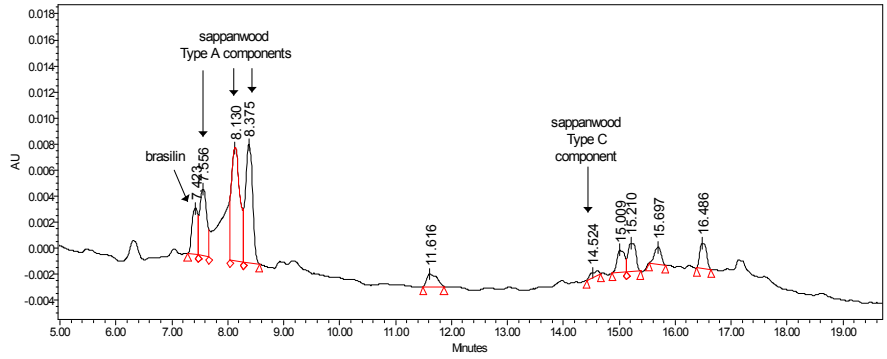
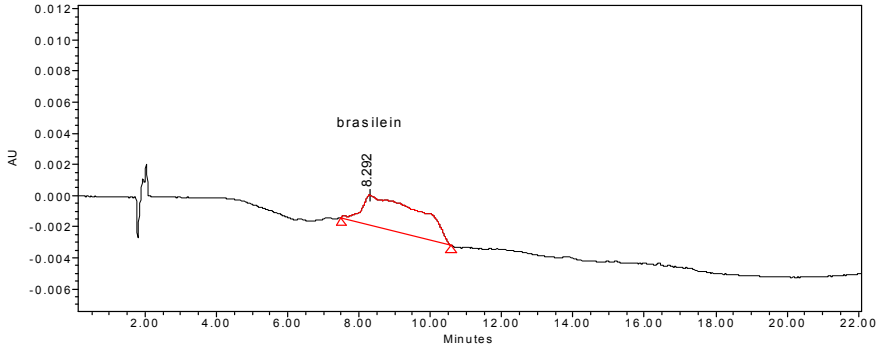


Fig. C.10. UHPLC-PDA chromatogram (monitored at 425 nm) of the smoketree dyed silk extract and UV-Vis spectra of its main constituents.



(a)



(b)

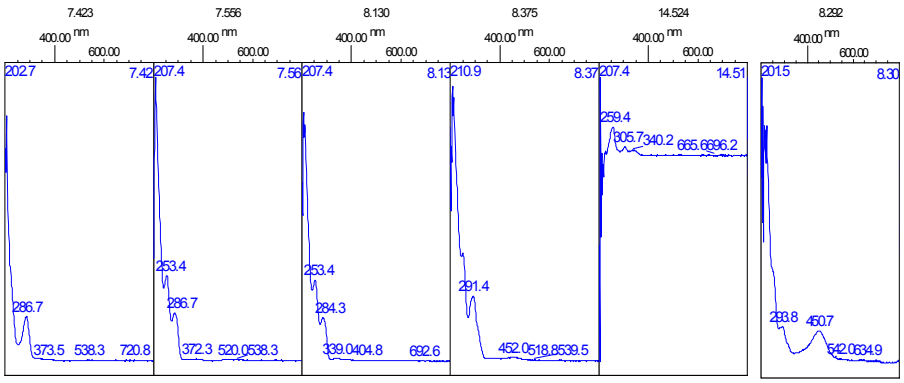


Fig. C.11. UHPLC-PDA chromatograms (monitored at (a) 295 nm and (b) 450 nm) of the sappanwood dyed silk extract and UV-Vis spectra of its main constituents.

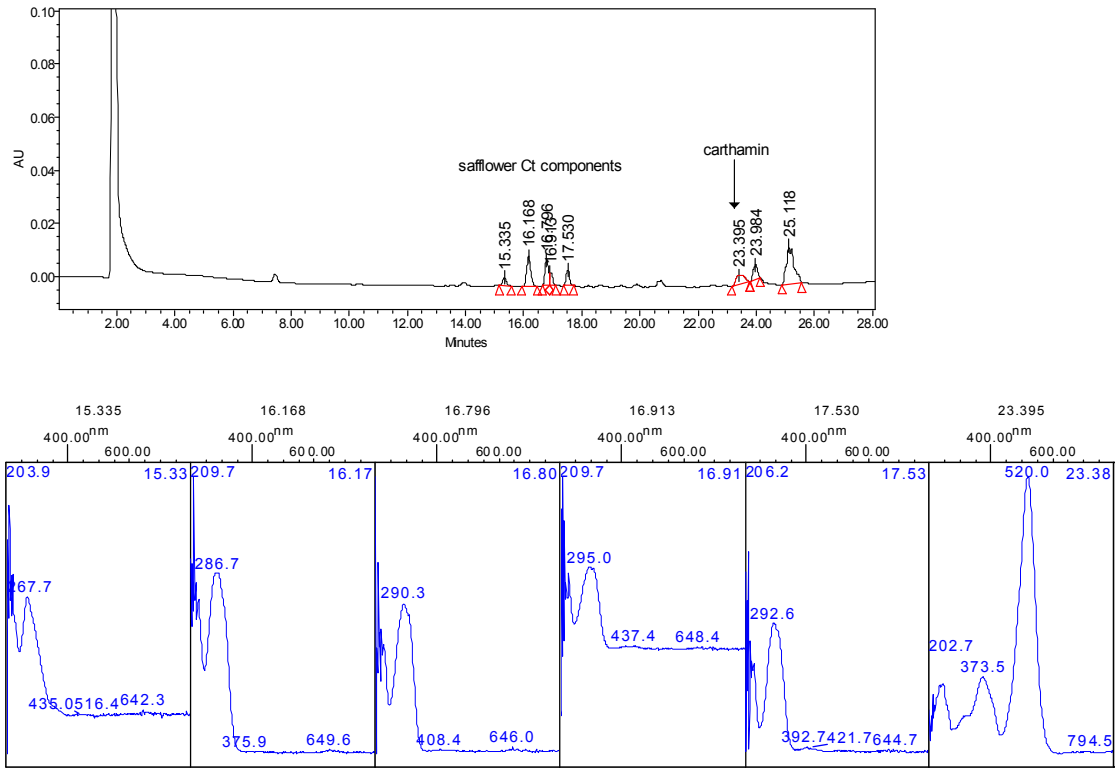


Fig. C.12. UHPLC-PDA chromatogram (monitored at 295 nm) of the safflower dyed silk extract and UV-Vis spectra of its main constituents.

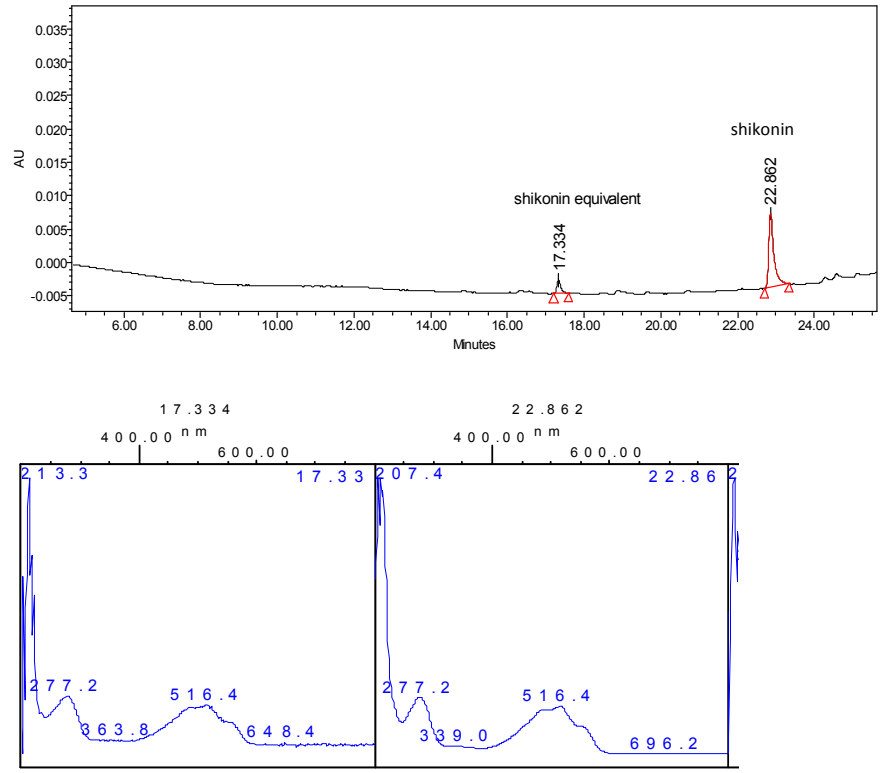


Fig. C.13. UHPLC-PDA chromatogram (monitored at 515 nm) of the gromwell dyed silk extract and UV-Vis spectra of its main constituents.

References

[1] W. Mullen, T. Yokota, M.E. Lean, A. Crozier, Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC–MS, Phytochemistry 64 (2003) 617-624.

[2] R. Singh, S. Chauhan, 9, 10-Anthraquinones and other biologically active compounds from the genus Rubia, Chem. Biodivers. 1 (2004) 1241-1264.